

**PERFORMANCE OF GRAMASREE BREEDER BIRDS FED
WITH DIFFERENT SOURCES OF CALCIUM**

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(19-MVP-26)

THESIS

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DECLARATION

I hereby declare that this thesis entitled **“Performance of Gramasree breeder birds fed with different sources of calcium”** is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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1. INTRODUCTION

In India, poultry farming was a backyard system until 1960s, and indigenous birds, despite their low productivity were utilised for egg and meat production purposes. The poultry farming has transformed over the past few decades, with indigenous birds being replaced by highly specialised and improved varieties. According to the 20th Livestock Census (2019), there were 317.07 million backyard poultry birds in India. On comparison with 19th Livestock Census (2012), there was a 46 per cent increase in backyard birds. In that scenario, Gramasree was developed by Kerala Agriculture University as an important germplasm which can increase the livelihood of backyard farmers. Gramasree was a synthetic cross of different breeds such as Barred Plymouth Rock, New Hampshire, Rhode Island Red and desi breeds which can be utilized in backyard farming.

Calcium is a necessary macro element for laying hens to produce egg and for optimal growth. Apart from that, 56 per cent of the motility-stimulating action of spermatozoa and seminal plasma of poultry depends on calcium (Ashizawa and Wishart, 1987) and their requirement also differs from female to male birds (Khalil *et al.*, 2012).

The egg production, fertility and hatchability of the laying hen depends on calcium and phosphorus content of the diet. Globally, 2.3 million tonnes of egg shell waste were generated during 2018 (Ahmed *et al.*, 2019) and estimated global egg production of 1,652 billion eggs (99 million tonnes) during 2019 (FAO, 2020), mainly from five leading egg-producing countries such as China, United States, Indonesia, India and Mexico which together contributed approximately 63 per cent of total global egg production. According to Abdulrahman *et al.* (2014) hen eggshell, a calcium-rich waste product from domestic sources such as hatcheries, poultry farms, fast food businesses, egg product manufacturers, households, and restaurants should be recycled, and diverted towards the creation of value-added goods to achieve sustainable development.

Egg shell contains around 98.2 per cent calcium carbonate, 0.9 per cent magnesium and 0.9 per cent phosphorus (phosphate), (Romanoff, and Romanoff, 1949). Eggshells as a by-product has the potential to be utilised as a calcium source (Olgun *et al.*, 2015; Tunç and Cufadar, 2015) in layer diets.

In commercial layers' ration, even though the calcium and phosphorus were balanced, still the layers were found to have a particular appetite for calcium (Newman and Sands, 1983). Various experiments have been designed to study the specific demand for calcium requirements and extra calcium source feeding techniques were followed to facilitate mechanical digestion at gizzard.

In India, limestone and oyster shells are used as calcium sources and mixed in feed. For grit feeding as free choice shell grits are also preferred in the commercial layer farms. Even though egg shells are available abundantly, their use as calcium sources are not practised and very few studies were conducted. Hence, considering the availability and use as a free-choice feeding calcium source in backyard poultry farming, the present study was designed with the following objectives,

1. To assess the effect of supplemented egg shell powder and shell grit on semen quality of Gramasree roosters.
2. To assess the effect of supplemented egg shell powder and shell grit on egg production, egg quality, fertility and hatchability of breeder hens.
3. Techno-economic of supplementing egg shell powder and shell grit on egg production.

2. REVIEW OF LITERATURE

In this chapter, the effect of different calcium sources in laying hens' diet were reviewed in terms of their source, particle size, inclusion level, feed efficiency, egg production performance, egg quality characteristics and male rooster's performance.

2.1 PRODUCTION PERFORMANCE IN IMPROVED VARIETIES OF NATIVE CHICKENS

Ramana *et al.* (2010) recorded an average first laying of eggs by Vanaraja as 181.2 days, the egg weight was 58.6 g at 40 weeks and the number of eggs laid up to 40th weeks was 52.8 eggs/ bird.

Rajkumar *et al.* (2014) evaluated the external egg quality characteristics of 198 eggs from 56 Aseel chickens at different weeks of age. They were given *ad-libitum* feeding with maize soya-based diets during the experimental period. At 32, 40 and 72 weeks, the mean egg weight was 37.08, 38.83, and 47.52 g, respectively, with an overall mean of 41.81 g.

Islam *et al.* (2014) reported that the mean age at first egg was 187.45 ± 1.02 days for Vanaraja and the average number of eggs were 33.13 ± 0.11 , 52.08 ± 0.32 , and 87.29 ± 1.02 , at 32, 40, and 52nd weeks, respectively.

Valavan *et al.* (2016) studied the Gramapriya birds production performance under an intensive system and reported that age at first egg was 175.52 days, hen day egg production was 50.60 per cent and egg weight at 40th weeks of age was 58.10 g. Meanwhile, the shape index of the Gramapriya egg was 77.22, albumen index was 0.71 ± 0.002 , Yolk index was 0.38 ± 0.003 , Haugh unit was 70.20 ± 1.20 and shell thickness (mm) was 0.32 ± 0.02 .

SenthilMurugan (2017) studied internal egg quality traits of different varieties of chicken housed in high altitude hilly regions from 44 to 48 weeks.

The Yolk Weight (g), Yolk Ratio (per cent), Yolk Height (mm), Yolk Weight (g), Yolk Length (mm), Yolk Index (per cent) and Albumen Height (mm) were 17.08 ± 2.08 , 31.63 ± 4.40 , 19.48 ± 0.49 , 42.03 ± 0.27 , 44.35 ± 0.84 , 45.11 ± 1.22 and 8.66 ± 1.14 , respectively in Gramasree birds.

Singh *et al.* (2018) recorded the production performance of improved varieties of native birds like Vanaraja and Gramapriya reared in backyard system at Jammu and Kashmir area for 60 weeks. It was recorded that the total number of eggs laid up to 40th weeks of age was 30.40 in Gramapriya and 22.20 in Vanaraja birds. Meanwhile, Gramapriya laid 100.87 eggs and Vanaraja birds laid 77.60 eggs up to 60 weeks. The average egg weight of Gramapriya was 53.07 g and Vanaraja was 53.53 g at 60th weeks of age.

Kumar *et al.* (2021) studied the geographical distribution of improved backyard varieties developed by ICAR-Directorate of Poultry Research and reported that Vanaraja and Gramapriya varieties as dual-purpose birds constituted about 52 and 38 per cent of backyard farming, respectively in India. Gramapriya was mostly liked in the Southern region (56%) followed by Central (25%) and North-Eastern (10.9 per cent) regions of India.

2.2 EFFECT OF CALCIUM SUPPLEMENTATION IN MALE BIRDS

2.2.1 Effect of calcium supplementation on feed intake and body weight

Chauhan *et al.* (2009) studied the physical and morphological features of 114 semen samples which were collected twice daily for 10 weeks from commercial dual-purpose Gramapriya hybrid cocks of 38 weeks using abdomen massage method. The average ejaculate volume was 0.39 ± 0.02 ml, with sperm concentration and total sperm count $2.49 \pm 0.03 \times 10^6$ / ml and $0.98 \pm 0.04 \times 10^9$ / ejaculate, respectively. Individual motility, live spermatozoa, and morphological aberrant spermatozoa were 84.01 ± 0.04 , 91.01 ± 0.29 , and 6.58 ± 0.22 per cent, respectively, while bulk motility was rated on a scale of 3.95 ± 0.03 . The pH of sperm was generally alkaline (7.41 ± 0.01).

Kanyinji and Maeda (2010) found that feeding dietary calcium at a 3.1 per cent level had shown enhanced seminal calcium level, sperm motility, thermo-tolerance and cryo-survivability in Barred Plymouth Rock roosters, however, at 5.4 per cent calcium level had shown a deleterious effect on sperm quality.

Gongruttananun (2011a) conducted a 10-week trial to investigate the effect of using ground eggshell and fine limestone as a calcium source in breeder male diets and reported that the feed intake was more in egg shell group (118.56 g /rooster/day) and the final body weight was 2.90 kg in control and 50 per cent egg shell group was 2.81 kg and 2.82 kg in 100 per cent egg shell group.

Namntu (2016) investigated the effect of feeding female ration to male broiler breeders and found that it had no influence on male fertility but it increased the male body weight.

Khalil *et al.* (2012) reported that cocks fed with two and three per cent calcium levels had superior forward sperm motility, live sperm and fertility per cent compared with those fed with one per cent calcium.

Lertchunhakiat *et al.* (2016) selected 15 Arbor Acers breeder cocks of 61 weeks and divided it into three treatments of five replicates each. The coarse limestone (LS) and fine LS at 5.52 per cent in control group (T1), T2 group with 5.52 per cent fine limestone and 5.52 per cent egg shell and T3 group with 10.8 per cent egg shell was fed. They reported that body weight of three groups T1, T2 and T3 were 4696.97, 4620.005, 5030.00 g, respectively and feed intake was 181.81, 187.04, 192.95 g/day, respectively in T1, T2 and T3. They indicated that egg shell powder inclusion at 10.8 per cent level did not influence the feed intake and body weight compared to other group cocks.

2.2.2 Effect of calcium supplementation on semen quality parameters

Gongruttananun (2011a) conducted a trial with supplementing egg shells in Rhode Island Red roosters and recommended that egg shells could be included

in breeder ration at 9.06 per cent in place of limestone powder without any difference in the semen volume (0.37 ml/ejaculate), sperm concentration (8.07 billion per ml) and sperm number per ejaculation.

Lertchunhakiat *et al.* (2016) selected 15 Arbor Acers breeder cocks, at 61 weeks of age were divided into three treatments of five replicates, and coarse LS and fine LS at 5.52 per cent each in control group (T1); T2 group with 5.52 per cent fine limestone and 5.52 per cent egg shell and in T3 group 10.8 per cent egg shell was fed. They had recorded that the mean semen volume of 0.39, 0.40 and 0.39 ml in T1, T2 and T3, respectively and the colour of the semen (milky white) revealed no significant difference among treatment groups. The mean sperm concentrations in T1, T2 and T3 groups were 157.64, 190.80 and 147.88 million/ml, respectively. The percentage of sperm motility of 76.27, 80.74 and 72.63 per cent in T1, T2 and T3, respectively and the results were similar among the treatment groups.

2.3 CALCIUM SOURCES FOR LAYER BIRDS

2.3.1 Significance of particle size and *in-vitro* solubility of calcium sources in laying hens

The particle size of the calcium sources influenced the bioavailability of calcium, while readily soluble fine particles were not available in the digestive tract of poultry at night hours for eggshell synthesis. The coarse and less soluble calcium sources were recommended to maintain adequate blood calcium level (Miles, 2000). It was explained by Junqueira & Rodrigues (2004) that coarse limestone as calcium source shown higher calcium retention period in the gizzard, and thus slow and uniform availability of calcium during eggshell synthesis results with higher eggshell weight and strength. Ito (2006) also added that the solubility rate of different calcium sources might be considered for better egg shell quality than particle size of the calcium sources.

Gongruttananun (2011b) studied the effect of using limestone (100 per cent), limestone and eggshell with limestone (50:50) and ground eggshell (100 per cent) in the diet of laying hens and reported that particle size distribution (amount in per cent) of ground eggshell used in the experiment with particle size of 6 to 10 mm was 0.54 per cent, 2 to 5 mm was 40.46 per cent and less than 2 mm was 59 per cent and it has shown better growth performance.

Pizzolante *et al.* (2011) studied the effect of different calcium sources with different particle size and solubility and reported that the particle size of fine limestone, coarse limestone and oyster shell in their study were 0.44, 2.40 and 2.19 mm, respectively. The *in-vitro* solubility values reported for fine limestone powder was 31 per cent and 28 per cent for coarse limestone and 46 per cent for oyster shell.

The dietary calcium requirement for laying hens were highly influenced by particle size, solubility and source of calcium in the feed and also supplemented as extra calcium source by choice feeding. The *in-vitro* solubility of limestone powder was reduced from 25.56 to 22.37 per cent when the particle size was increased from 0.18 mm to 3.90 mm (Pelicia *et al.*, 2011).

Ahmed *et al.* (2013) conducted an experiment in Bovans layers by supplementing limestone powder and oyster shell as calcium source. The calcium content of limestone powder and oyster shell was 39.9 and 39.5 per cent, respectively. They found that limestone supplementation at 6 per cent level had shown significantly ($p < 0.05$) better egg production, feed intake and feed conversion efficiency compared to oyster shell. However, no changes were reported in egg shell quality, but rate of lay was significantly lower in oyster shell (59.82 per cent), compared to lime stone (76.19) group.

Olgun *et al.* (2015) reported that the calcium, phosphorus and magnesium content of eggshell powder as 32.3, 7.6 and 9.16 per cent respectively. Similarly, calcium, phosphorus and magnesium content of limestone powder was 33.97, 0.13 and 4.36 per cent, respectively. The oyster shell contained 32.09, 1.09 and 9.08

per cent of calcium, phosphorus and magnesium, respectively. They had given 4.5 per cent of calcium to 72 Hy-Line W36 layer birds at 25 weeks age and one third of the limestone quantity in control group ration was replaced by egg shell powder.

Lertchunhakiat *et al.* (2016) studied the effects of different calcium sources such as coarse limestone (5.52 per cent in diet), fine limestone (5.52 per cent in diet) and grounded eggshell (10.8 per cent in diet) at different combinations of coarse, fine limestone and grounded eggshell in 15 Arbor Acres breeder cocks diet at 61 weeks and reported that the grounded egg shell as calcium source in breeder cock diet shown similar results with limestone supplementations without affecting normal semen quality.

Saki et al. (2019) studied the influence of oyster shell supplementation with different particle size (<1 mm, 1-2 mm and 3-4 mm) as calcium source with solubility of 57.69, 51.95 and 45.83 per cent, respectively in 200 leghorn layers at 40-week and found that 50 per cent oyster shell with 2-4 mm particle size and 50 per cent of fine oyster shell powder had improved the physical quality of eggs.

Rathnayaka *et al.* (2020) measured the particle size and solubility of different calcium sources supplemented to commercial layers and the particle size distribution of calcium sources was measured as geometric mean diameter (GMD) and geometric standard diameter (GSD). They reported that the GMD of oyster shells, limestone and bone meal as 0.616, 2.23 and 0.465 mm, respectively. Similarly, GSD of oyster shells, limestone and bone meal were 2.44, 2.18 and 2.40, respectively. There was higher proportion of coarse particles (>2.3 mm) measured in limestone (58.53 per cent). Medium sized particles (0.354 – 2.38 mm) were higher in oyster shells (56.01 per cent). More fine particles (0.225 mm or less - 52.80 per cent) were present in the bone meal compared to other two sources.

Lee *et al.* (2021) conducted an experiment to compare the effect of different calcium sources with different particle size including limestone (< 2 mm

and 2–4 mm mixed in the ratio of 3:7), cockle shell (1–4 mm), oyster shell (3–16 mm), egg shell fine particles (< 1 mm) and egg shell coarse particles (3–5 mm) in 450 ISA-Brown laying hens at 73 weeks. The egg shell membranes were removed by washing with water, then heated at 150° C for 12 hours and crushed to required particle size. The different calcium sources were distributed daily around 10 am. The results obtained in the study indicated coarse egg shell powder shown significant improvement on egg quality.

2.4 EFFECT OF DIFFERENT SOURCES OF CALCIUM SUPPLEMENTATION IN LAYER BIRDS

2.4.1 Effect of calcium supplementation on feed intake and feed efficiency

Gongruttananun (2011c) studied the effect of using ground egg shell and fine limestone as a calcium source on body weight by feeding T1, T2 and T3 experimental diets and found that there was no significant change in final body weight of 1.78, 1.83, 1.89 kg, respectively among the treatment groups T1, T2 and T3. The feed intake in T1, T2 and T3 fed groups were 116.82, 118.13 and 123.33 g/day per hen, respectively and similar results were found the between treatment groups.

Pelicia *et al.* (2011) investigated the different calcium levels (limestone as dietary calcium source) such as 3.5 (T1), 3.75 (T2), and 4.5 (T3) per cent with three particle sizes (fine, mix of fine and coarse and coarse) and reported that the calcium source did not influence the feed intake and feed efficiency to produce 12 eggs in ISA-brown layers. The feed intake recorded in the study was 132.6 g, 133.4 g and 130.2 g/hen/day in T1, T2 and T3 groups, respectively. The feed conversion ratio for dozen eggs calculated in the study was 1.74 (T1), 1.769 (T2) and 1.77 (T3).

Pizzolante *et al.* (2011) evaluated the effect of different dietary calcium sources (limestone and oyster shell) and levels supplemented at 2.6, 3.2 and 3.8 per cent with different particle size (fine, coarse, mix of coarse and fine) in 576

Hy-Line brown layers on the egg production, feed efficiency and egg quality. The results reported that feed intake and feed conversion efficiency to produce dozen eggs and kg egg mass were similar between the groups and the average feed intake was 119.83 g/bird/day, feed conversion ratio for 12 eggs and per kg eggs were 1.79 and 2.10, respectively.

Houndonougbo (2012) conducted an experiment with supplementing oyster shell and snail shell in 72 ISAs Brown laying hens of 60 to 72nd weeks which were assigned to four dietary groups. The total balanced feed intake was significantly lesser in snail shells supplemented group (99.0 g/ hen) and feed conversion efficiency (kg feed/ kg egg) was poorer in snail shell supplemented group compared to oyster group.

The inclusion of egg shell waste as calcium source in ISA-Brown hens' diet, fed for 12 weeks of experimental period shown significant increase in feed, calcium and phosphorus intake in 7.5 per cent egg shell waste fed group and the values were 116.81 g, 4.02 g and 0.64 g/hen/day, respectively on comparison to other groups fed with lesser quantity of egg shell waste (Kismiati *et al.*, 2012).

Pastore *et al.* (2012) conducted an experiment in Hy-Line W-36 laying hens at the post-laying between 42nd and 58th weeks and the diet consist of three levels of calcium (39, 42, and 45 g/kg diet) and three calcium: phosphorus ratios (12.12:1, 10.53:1, and 9.30:1) and reported that the feed intake was not affected by different levels of calcium intake and difference in calcium: phosphorus ratio of the diet.

Ahmed *et al.* (2013) explored the impact of feeding oyster shell and limestone powder in 24 Bovans laying hens which were grouped as G1(oyster shell) and G2 (limestone powder) with 12 birds in each treatment group and four replicates each, with 3 birds in each replicate. There was significant increase in feed intake in G2-limestone group (142.02 g/hen/day) compared to G1-oyster shell group (139.53 g/hen/day) at 24th week. The feed efficiency in G2 group

(2.99) was significantly better than G1 (4.66) group during the experimental period.

Olgun *et al.* (2015) conducted a feeding trial with supplementing egg shell powder and oyster shell replacing at 1/3rd and 2/3rd of limestone content in Hy-Line W36, layer diet at 25 weeks and found that the feed intake was 94.42 and 94.54 g/hen/day, when 2/3rd of limestone was replaced by oyster shell and egg shell powder, respectively, compared to control group (100.50 g/hen/day) birds supplemented with 100 per cent limestone powder. However, feed efficiency recorded (g feed /g egg) was similar in all groups and ranged between 1.79 to 1.89.

Lee *et al.* (2021) conducted an experiment with supplementing different calcium sources *viz.*, limestone, cockle shell, oyster shell, fine egg shell and coarse egg shell in layer diet and reported that feed intake was 106.3 g, 107.0 g, 105.6 g, 106.4 g and 106.5 g, respectively in different groups. The supplementation of egg shell instead of limestone powder did not affect the feed intake and similar results were found between the other treatment groups.

2.4.2 Effect of calcium supplementation on calcium intake and body weight of layers

Ahmed *et al.* (2013) explored the impact of feeding oyster shell and limestone powder in 24 Bovans laying hens assigned as G1-oyster shell and G2-limestone powder group with 12 birds in each treatment with four replicates, with 3 birds in each replicate. At the end of the experiment, the birds received limestone powder as calcium source shown positive change in body weight (8.91 g) compared to oyster shell supplementation shown negative change in body weight (-4.67 g)

Olgun *et al.* (2015) found supplementation of egg shell powder and oyster shell had no significant change in body weight of the laying hens during the experimental period from 25th -37th weeks and maximum level of 9.28 per cent of

egg shell was included in Hy-line laying hens, without affecting their feed intake and calcium intake during the experiment.

Swiatkiewicz *et al.* (2015) evaluated the effect of dietary level and particle size of dietary calcium on egg production in ISA-Brown hens from 25 to 70 weeks of age. The birds were fed with three different calcium levels at 3.20, 3.70, and 4.20 per cent, at 0, 25.0 and 50.0 per cent substitutions of limestone with particle sizes of 0.2–0.6 mm and 1.0–1.4 mm. During 25th-40th weeks, the mean daily feed intake, feed conversion in all treatment groups was 113 g/hen and 1.97 kg of feed per kg eggs, respectively. Like that, during 41 to 70th weeks the mean daily feed intake in all treatment groups was 117 g/hen and feed conversion efficiency in all treatment groups was 2.06 kg of feed/kg of egg.

Rathnayaka *et al.* (2020) studied the effects of free-choice feeding of oyster shell, limestone and bone meal as calcium sources and reported that they were not significantly affecting the body weight with weight gain of 35, 35 and 45 g/hen/day in oyster shell (T1), limestone (T2) and bone meal (T3), respectively. The calcium intake recorded was 5.96, 6.28 and 4.62 g/bird/ day for T1, T2 and T3, respectively and the feed efficiency for 12 eggs was 1.30, 1.32 and 1.29, respectively in T1, T2 and T3 groups.

2.4.3 Effect of calcium supplementation on egg production

The hen day and hen housed egg production (numbers of egg) in improved varieties of native chicken recorded in Gramalakshimi was 42.28 and 38.63 and Gramapriya layers was 40.32 and 38.60, respectively. Like that, hen day and hen housed egg production (per cent) were recorded as 35.53 and 32.46 per cent for Gramalashimi laying hens and 34.3 and 32.44 per cent in Gramasree laying hens (Ajithbabu, 2010).

Pelicia *et al.* (2011) studied the effect of different dietary calcium levels at 3.50, 3.75 and 4.50 per cent in ISA brown layers which are distributed into nine treatments, with nine replicates of five birds in each replicate with three limestone

particle sizes of fine limestone (100 per cent fine limestone, FL); 70 per cent FL + 30 per cent coarse limestone (CL) and 50 per cent (FL) + 50 per cent CL. The findings indicated that 5.21 g/day/hen calcium intake reduced the egg production to 87.6 per cent, compared to 3.98 g/day/hen intake group and recorded 91.3 per cent of egg production. The particle size had not significantly influenced the egg production.

Gongruttananun (2011c) supplemented egg shell powder as calcium source in 144 Rhode Island Red hens at 25 weeks of age in place of limestone powder at different combinations. The birds housed in G1 group received limestone (100 per cent), in G2 group limestone and eggshell was mixed at 50:50 per cent and G3 group birds received ground eggshell (100 per cent). The egg production reported in G1, G2 and G3 was 61.18, 69.24 and 68.28, respectively and the results were similar in all the treatment groups.

Houndonougbo (2012) conducted an experiment with supplementing oyster shell and snail shell, 72 ISA Brown laying hens 60 to 72nd weeks were assigned to four dietary groups, 18 hens in nine cages and two hens each. The egg production was significantly lesser in snail shell supplemented group (80.6 per cent) compared to oyster shell (85.3 per cent) supplemented group.

Kismiati *et al.* (2012) conducted experiment in ISA Brown laying hens for 12 weeks of experimental period from 25th weeks. In this experiment, limestone and egg shell were used as calcium sources and supplemented in the diet at different combinations of 7.5 per cent limestone as a control, 5 per cent limestone + 2.5 per cent egg shell, 2.5 per cent limestone + 5 per cent egg shell, and 7.5 per cent eggshell. The results of this study revealed that egg production and egg weight in 7.5 per cent egg shell fed group shown significantly increased egg production (91.42 per cent) and egg weight (55.73 g/egg) and increase in feed, protein, calcium and phosphorus intake was reported in the study might be the reasons for increase in egg production.

Ahmed *et al.* (2013) explored the impact of feeding oyster shell and limestone powder in 24 Bovans laying hens which were assigned as G1-oyster shell and G2-limestone powder supplemented group with 12 birds in each treatment with four replicates, with 3 birds in each replicate. There was significantly better egg production of 76.19 per cent was recorded in G2 compared to G1-oyster shell group (59.82 per cent).

Olgun *et al.* (2015) recorded the egg production of 72 Hy-Line W36 layer birds, distributed in six experimental groups, with four replicates, and three birds in each replicate. Six different layer rations were prepared with egg shell, oyster shell replacing limestone powder *viz.*, T1-Control (limestone), T2-1/3 eggshell, T3- 1/3 oyster shell, T4-1/3 eggshell + 1/3 oyster shell, T5- 2/3 eggshell and T6- 2/3 oyster shell and fed from 25th-35th weeks of age. The egg production recorded in T4 was 93.85 egg/100 birds/day which was significantly more than T2 (87.70) and T3 (86.90) group birds and there is no significant difference in T1 (90.63), T5 (90.48) and T6 (90.03) group birds.

Swiatkiewicz *et al.* (2015) evaluated the effect of dietary level and particle size of dietary calcium on egg production in ISA Brown hens from 25 to 70 weeks of age. The birds were fed three different calcium levels at 3.20, 3.70, and 4.20 per cent, at 0, 25.0 and 50.0 per cent substitutions of limestone with particle sizes of 0.2–0.6 mm and 1.0–1.4 mm. During 25th-40th weeks, the mean egg weight, daily egg mass/hen, and egg production of all dietary groups recorded were 59.9 g, 57.3 g, and 95.5 per cent, respectively. Like that, during 41st to 70th weeks, the mean (all dietary groups) egg weight was 62.4 g, daily 57.7 egg mass/hen, and 92.0 per cent of egg production. The results indicated that excess or reduction in 3.20 per cent of calcium level did not influence the egg production performance of laying hens from 25th-70th weeks of age and optimal level of 3.20 per cent was sufficient.

An *et al.* (2016) conducted an experiment with 500, 70-wk-old Hy-Line Brown, were divided into five dietary groups with 10 replicates and 10 birds in

each replicate, the calcium was fed at 3.5, 3.8, 4.1, 4.4 and 4.7 per cent by supplementation of limestone powder. Egg production (per cent) recorded were 75.10, 76.00, 75.20, 75.00 and 79.10 in 3.5, 3.8, 4.1, 4.4 and 4.7 per cent calcium, respectively in calcium fed group, no significant results were found in egg production level as their calcium content of the feed increased and no increase in feed intake were also recorded in this experiment. Like that, egg weight (g/egg) weighed was 60.4, 60.3, 61.0, 60.8 and 61.2 in 3.5, 3.8, 4.1, 4.4 and 4.7 per cent calcium fed groups, respectively.

Salajegheh *et al.* (2020) conducted feeding experiment in 192, 94-weeks Bovanz white commercial layers which were allotted into six dietary groups with four replicates of eight birds each, with different calcium sources such as limestone A, limestone B, and oyster shell. They reported that the egg production (per cent) 82.10, 82.30 and 83.42, respectively in limestone A, limestone B, and oyster shell fed groups.

Lee *et al.* (2021) studied the effect of using different calcium sources such as limestone, cockle shell, oyster shell, fine egg shell and coarse egg shell on egg weight in brown laying hens at 73 weeks and reported that the average egg weight (65.93 g) and daily egg mass (37.64 g/hen/day) was significantly increased in coarse egg shell group. They also reported that lightest and heaviest eggs were produced in groups fed with cockle shell and coarse egg shell groups, respectively.

2.5 EFFECT OF CALCIUM SUPPLEMENTATION ON EGG QUALITY

2.5.1 External egg quality parameters

Ahmed *et al.* (2013) explored the impact of feeding two different calcium sources (oyster shell and limestone) to Bovan layer chickens and reported that calcium source had no influence on egg weight (51.52 and 54.08 g, respectively among groups), egg height (54.60 and 65.11 mm, respectively among groups) and

egg diameter (41.43 and 41.95 mm, respectively among groups) by supplementing oyster shell and limestone.

Sinha *et al.* (2017) estimated genetic effect on internal egg qualities of Vanaraja (dual purpose type) and Gramapriya (the layer type) and their crosses on random mating. The egg length, width, shape index, shell thickness, shell per cent, and shell weight of Gramapriya were 51.66 mm, 40.10 mm, 77.719, 0.37 mm, 17.925 per cent and 4.458 g, respectively. The reported values for Vanaraja layers in the study were egg length (52.04 mm), width (40.29 mm), shape index (77.49), shell thickness (0.36 mm), shell per cent (17.266 per cent), and shell weight (4.147 g).

Rathnayaka *et al.* (2020) investigated the impact of free-choice feeding of three different calcium sources such as oyster shell (OS), lime stone (LS) and bone meal (BM) to commercial layers and reported egg weights were measured at 60.70 (OS), 61.63 (LS) and 61.90 g (BM), respectively among treatment groups. The shape index was 76.41 (OS), 76.44 (LS) and 75.60 (BM) per cent respectively among treatment groups.

Lee *et al.* (2021) studied the effect of using different calcium sources (Limestone, cockle shell, oyster shell, fine egg shell and coarse egg shell) on egg weight in brown laying hens at 73 weeks and reported that the average egg weight was 65.93 g and daily egg mass was 37.64 g/hen/day in coarse egg shell group, which significantly more than other groups. They also reported that lightest and heaviest eggs were produced in groups fed with cockle shell and coarse egg shell groups respectively.

2.5.2 Internal egg quality parameters

Gongruttananun (2011c) conducted a study in laying hens and supplemented with limestone powder in G1, egg shell was supplemented replacing 50 per cent of limestone powder in G2 and in G3 group, 100 per cent egg shell powder in place of limestone powder as source of calcium source in RIR

laying hens and found that egg shell powder as dietary calcium source replacing limestone powder, up to 9.06 per cent did not affect egg production and internal quality characteristics of egg. The albumen (per cent), albumen height (mm), yolk weight (per cent), yolk colour (Roche scores) recorded in their study were 65.0 per cent, 6.62 mm, 25.46 per cent and 5.94, respectively in G3 groups. Similarly, egg specific gravity recorded was 1.084, 1.084 and 1.083 in G1, G2 and G3 groups, respectively.

Ahmed *et al.* (2013) explored the impact of feeding two different calcium sources (oyster shell and limestone) to layer chickens (Bovan) and reported that calcium sources had no influence on albumen weight (28.77 and 31.05 g, respectively in treatment groups), albumin height (6.37 and 6.36 mm, respectively in treatment groups) and yolk weight (16.46 and 16.46 g, respectively in treatment groups)

Sinha *et al.* (2017) estimated genetic effect on internal egg quality characters of Vanaraja (dual purpose type) and Gramapriya (the layer type) and their crosses on random mating. They reported the Gramapriya eggs internal qualities such as albumen height, albumen index, Haugh Unit, yolk height, yolk width, yolk index, albumen weight, yolk weight, albumen weight and yolk weight as 5.691mm, 7.396 mm, 79.38, 15.464 mm, 35.513 mm, 43.102 per cent, 28.513 g, 13.574 g, 51.476 per cent and 32.745 per cent, respectively. Like that, they had mentioned Vanaraja eggs internal qualities of albumen height as 5.87 mm, albumen index as 7.802, Haugh Unit as 81.35, yolk height as 16.44 mm, yolk width as 37.464 mm, yolk index as 43.88 per cent, albumen weight as 30.011 g, yolk weight as 12.692 g, albumen weight as 53.156 per cent and yolk weight as 31.324 per cent.

Rathnayaka *et al.* (2020) performed a study in 128 (55 weeks old) Hy-line white layers which were individually assigned to 16 cages of 8 birds each. They fed commercially available layer feed with oyster shell (T1), limestone (T2) and bone meal (T3). The internal egg quality characteristics such as albumen index

was 9.93, 9.86 and 9.72, respectively in T1, T2 and T3. The albumen ratio was 62.9, 61.6 and 62.3 in T1, T2 and T3, respectively. The Haugh unit calculated in T1, T2 and T3 was 86.23, 86.45 and 85.98, respectively among treatment groups. The yolk colour was 12.02 (T1), 12.09 (T2) and 12.03 (T3), yolk index was 43.53 (T1), 43.60 (T2) and 43.72 (T3) and yolk ratio was 27.61(T1), 28.85 (T2) and 27.67 (T3), respectively among treatment groups. Calcium supplementation had no effect ($p>0.05$) on internal egg quality parameters by feeding different calcium sources.

Lee *et al.* (2021) evaluated the effect of using different calcium sources (Limestone, cockle shell, oyster shell, fine and coarse egg shell) on internal egg quality and reported that the eggs from oyster shell and coarse egg shell fed hens showed increased albumen height (7.65 and 7.62 mm respectively) in comparison to eggs from cockle shell (7.14 mm) group ($p < 0.05$). The yolk colour (5.97) was darker in the fine egg shell group as compared with other dietary groups ($p < 0.01$). However, there was no significant difference on Haugh units (84.85, 83.60, 86.06, 84.90 and 85.98, respectively) among the treatment groups ($p > 0.05$).

2.5.3 Egg shell quality parameters

Pelicia *et al.* (2009) investigated the effect of four calcium levels and four available phosphorus levels in the diet of Hisex Brown layers between 90th-108th weeks after molting. The experimental diet consists of 16 treatments with different Ca and phosphorus levels. Four groups with 3.0 per cent Ca and 0.25 per cent P (T1), 0.30 per cent P (T2), 0.35 per cent P (T3), 0.40 per cent P (T4). Then another four groups with 3.5 per cent Ca and 0.25 per cent P (T5), 0.30 per cent P (T6), 0.35 per cent P (T7), 0.40 per cent P (T8), then another four groups with 4.0 per cent Ca and 0.25 per cent P (T9), 0.30 per cent P (T10), 0.35 per cent P (T11), 4.0 per cent Ca and 0.40 per cent P (T12), then another four groups with 4.5 per cent Ca and 0.25 per cent P (T13), 0.30 per cent P (T14), 0.35 per cent P (T15) and 0.40 per cent P (T16). They found that dietary calcium levels influenced

eggshell per cent (9.53 per cent) and eggshell weight per surface area (ESWSA) of 84.90 mg/cm² measured at 4.5 per cent calcium supplemented diet.

Gongruttananun (2011c) divided 144 Rhode Island Red layers at 25 weeks of age into three groups with four replicates with 12 birds in each replicate, provided with a layer ration supplemented with limestone powder in G1(100 per cent), egg shell was supplemented replacing 50 per cent of limestone powder in G2 and G3 birds with 100 per cent egg shell powder which was supplemented in place of limestone powder as calcium source. They had reported that supplementation of egg shell powder did not influence the egg shell weight (8.93, 8.89 and 8.83 per cent in G1, G2 and G3 groups, respectively). Like that, shell thickness in G1, G2 and G3 groups were 0.333 mm, 0.329 mm and 0.325 mm, respectively.

Pizzolante *et al.* (2011) studied the effect of different calcium sources and recommended that, at 3.8 per cent dietary calcium was supplemented as 50 per cent fine limestone and 50 per cent coarse limestone powder or oyster shell shown better egg shell quality in brown layers.

Houndonougbo (2012) conducted an experiment with supplementing oyster shell and snail shell in 72 ISA Brown layers from 60 to 72nd weeks of age and were assigned to four dietary groups, 18 hens in nine cages and two hens each. The eggshell weight (6.22 g) and egg shape index (75.6 per cent) was significantly lesser in snail shell supplemented group. The reported values of shell index, egg shell thickness, egg shell percentage for snail shell were 8.34 g/cm², 0.367 mm, and 9.78 per cent, respectively.

Ahmed *et al.* (2013) explored the impact of feeding oyster shell and limestone to layer chickens (Bovan) and reported that calcium sources had no influence on shell weight (5.21 and 5.45 g), shell thickness (0.28 and 0.27 mm), or shell ash (90.65 and 92.14 per cent) between the two treatments.

Olgun *et al.* (2015) recorded the egg production of 72 Hy-Line W36 layer birds, distributed in six experimental groups, with four replicates, and three birds in each replicate. Six different layer rations were prepared with egg shell, oyster shell replacing limestone powder *viz.*, T1-Control (limestone), T2-1/3 eggshell, T3- 1/3 oyster shell, T4-1/3 eggshell + 1/3 oyster shell, T5- 2/3 eggshell and T6- 2/3 oyster shell and fed from 25th-35th weeks of age. The egg shell thickness, egg shell weight and egg breaking strength reported was ranged from 0.352 to 0.371 mm, 9.03 to 9.64 g/100 g egg and 4.02 to 4.24 kg, respectively. All other values except egg shell weight were significantly more in 1/3rd of oyster shell supplemented group and lesser in 2/3rd oyster shell supplemented group, compared to other groups.

Swiatkiewicz *et al.* (2015) evaluated the effect of dietary level and particle size of dietary Ca supply on egg production quality in ISA Brown hens from 25th to 70th weeks of age and reported that the partial (25 or 50 per cent) substitution of fine-particle (diameter 0.20–0.60 mm) with larger-particle limestone (1.00–1.40 mm) improved egg shell per cent, shell thickness, density and breaking strength in aged layers irrespective of calcium dietary level.

Bingfan *et al.* (2017) explained that the shell weight per unit surface area and specific gravity of egg was significantly increased at higher intake of dietary calcium at 5.0 g/day/hen where limestone solubility was lesser and reported specific gravity as 1.0765 and shell weight unit surface area as 70.25 mg/cm².

Rathnayaka *et al.* (2020) investigated the impact of providing free-choice feeding of three different calcium sources to commercial layers and found the average shell ratios of the birds fed oyster shell, limestone and bone meal and control were 9.44, 9.60, 10.01 and 9.19 per cent, respectively. The unit surface shell weight (USSW) was 66.08, 65.84 and 70.30 mg/cm² in oyster shell, limestone and bone meal supplemented groups. The highest mean USSW was given by the birds fed bone meal (70.3 mg/cm²) compared to those fed oyster shell, limestone and control. Egg shell thickness of 0.537, 0.500 and 0.597 mm,

were measured in oyster shell, limestone and bone meal, respectively was significantly different among the treatment groups. Birds fed bone meal reported the highest shell thickness of 0.597 mm and oyster shell fed groups was 0.537 mm, significantly differ from other calcium sources.

Lee *et al.* (2021) studied the effect of using different calcium sources namely limestone (T1), cockle shell (T2), oyster shell (T3), fine egg shell (T4) and coarse egg shell (T5). They reported that the eggshell breaking strength was 3.62, 3.79, 3.72, 3.76 and 3.76 kgf, respectively among treatment groups. Like that, eggshell thickness of 0.392, 0.393, 0.393, 0.383 and 0.389 mm, respectively among treatment groups and no significant difference were found between the different sources of calcium.

2.6 EFFECT OF CALCIUM SUPPLEMENTATION ON TIBIAL AND SERUM CALCIUM CONTENT

Pelicia *et al.* (2009) investigated the effect of different dietary calcium levels (3.5, 3.75, and 4.5 per cent) and three limestone particle sizes such as 100 per cent fine limestone (FL), 70 per cent FL + 30 per cent coarse limestone (CL), and 50 per cent (FL) + 50 per cent (CL), and found that the blood Ca level (14.98, 17.09, 17.59 and 18.92 mg/dL) increased as dietary Ca level increases and the blood Ca level numerically decreased as the ratio of coarse limestone increased.

Gongruttananun (2011c) divided 144 Rhode Island Red laying hens at 25 weeks of age into three groups with four replicates with 12 birds in each replicate, provided with a layer ration supplemented with LS powder in G1, egg shell was supplemented replacing 50 per cent of LS in G2 and in G3 birds 100 per cent egg shell powder was supplemented in place of LS as source of calcium. The tibial bone weighed in experimental birds was 13.07 g, 13.13 g and 14.46 g in G1, G2 and G3 groups, respectively. The tibial weight (g/kg of body weight) was 7.01, 7.99 and 8.10 and ash content estimated was 54.10, 52.10 and 54.19 per cent in G1, G2 and G3 groups, respectively. The tibial bone breaking strength recorded was 27.39, 23.37 and 24.84 kg in the experimental birds in G1, G2 and G3

groups, respectively. The serum calcium measured in G1, G2 and G3 groups were 19.56, 16.13 and 16.20 mg/dl, respectively. Similarly, the phosphorus content measured was 8.23, 9.63 and 8.40 mg/dl in G1, G2 and G3 groups, respectively.

Pelicia *et al.* (2011) studied the effect of different dietary calcium levels of 3.5, 3.75, and 4.5 per cent in ISA brown layers distributed into nine treatments, with nine replicates of five birds in each replicate with three limestone particle sizes of fine limestone (100 per cent fine, FL); 70 per cent FL + 30 per cent coarse limestone (CL) and 50 per cent (FL) + 50 per cent CL. They found that tibial calcium content varies with dietary calcium levels (0.171 per cent at 3 and 3.75 per cent Ca, and 0.168 per cent at 4.5 per cent Ca). The serum calcium level of 228 mg/L, 215 mg/L and 227 mg/L were estimated in 3.0, 3.75 and 4.5 per cent calcium supplemented group at 35th weeks of age. The excess intake of calcium (5.9 g/day) might be excreted through faeces.

An *et al.* (2016) estimated the calcium content of blood and tibia in 70-wk-old Hy-Line Brown layers supplemented with graded level of calcium (3.5, 3.8, 4.1, 4.4, or 4.7 per cent) and reported that tibial calcium content of 17.79 to 18.25 per cent and serum calcium level of 27.2 to 29.0 mg/dL. Like that, phosphorus content of tibia was ranged from 8.55 to 9.23 per cent and 5.99 to 6.55 mg/dL.

Lee *et al.* (2021) evaluated the effect of limestone (T1), cockle shell (T2), oyster shell (T3), fine egg shell (T4) and coarse egg shell (T5) as calcium sources in layer diet and reported that the tibial characteristics such as tibial weight of 12.33, 11.83, 11.98, 11.92 and 12.31 g, respectively among treatment groups. The tibial length measured as 118.0, 116.8, 118.7, 117.7 and 118.4 mm and the width as 7.20, 7.13, 7.47, 7.67 and 7.33 mm, respectively measured among treatment groups. The breaking strength was reported as 23.95, 23.97, 23.12, 23.95 and 25.47 kgf respectively, among treatment groups. The tibial characteristics were non-significant among the different calcium supplemented groups. They also reported that there was no significant effect on Ca to P ratio in the serum of hens

fed different dietary calcium sources during light and dark periods of the experiment.

2.7 EFFECT OF CALCIUM SUPPLEMENTATION ON REPRODUCTIVE PERFORMANCE OF LAYER BIRDS

2.7.1 Fertility and Hatchability (per cent)

Gongruttananun (2011b) conducted a trial with supplementing egg shells in Rhode Island Red roosters and reported that there were no significant effects on fertility (96.44, 97.51 and 96.18 per cent in T1, T2 and T3, respectively) and hatchability (70.29, 76.37 and 77.10 per cent T1, T2 and T3, respectively) with respect to calcium source.

Kumar *et al.* (2012) studied the fertility and hatchability characteristics of improved varieties of Black Rock, Gramapriya, and Vanaraja layers and found that the mean fertility per cent was 87.96 per cent in Black Rock, 87.22 per cent in Gramapriya, and 87.78 per cent in Vanaraja. The overall mean hatchability on total egg set (TES) and fertility egg set (FES) basis of Black Rock was 63.52 and 72.21 per cent, Gramapriya was 70.93 and 81.32 per cent, and Vanaraja was 71.67 and 81.65 per cent.

Rajkumar *et al.* (2011) evaluated the reproductive, growth, and production performance of naked neck broiler breeders during a 72-week production cycle. Fertility and hatchability per cent in bareneck hens were 81.09, 82.14 (FES), and 75.46 (TES), respectively. Egg weight varied from 53.20 g from 28 weeks to 70.45 g at 72 weeks, with low to moderate heritability. Part-period egg production during 40 weeks was 64.18, with an annual yield of 164.37 eggs. The correlation coefficients revealed a strong positive and significant link between body weights at different ages, as well as a negative relationship between body weights and egg weights.

Kumar *et al.* (2016) performed a survey to describe the behaviour, mortality pattern, and egg production of Kerala's indigenous chicken. The body weight of male bird at 20th and 40th week body weights were reported as 1,428.42 and 1,936.67 g and female body weight as 1,114.04 and 1,445.63 g, respectively. The fertility rate of 71.22 per cent, hatchability of 62.26 per cent on total egg set and 87.42 per cent on fertile egg set, respectively were recorded. The average age at sexual maturity and the age at which the first egg was laid were 155 and 199.26 days, respectively and incubation pause and broodiness were recorded as 26.03 and 121.75 days, respectively.

Patra *et al.* (2016) conducted a study to investigate the effect of egg weight on fertility and hatchability in Vanaraja and Gramapriya birds. Eggs from the Vanaraja (592) and Gramapriya (657) birds were weighed and set into G1 (big size, >65 g), G2 (middle size, <65 g), and G3 (small size, 65 g) and incubated in three batches. Fertility, hatchability, and chick weight were all affected by the egg size. Medium-sized eggs had the highest degree of fertility, followed by big and small-sized eggs. On total egg set medium-sized eggs had a greater hatchability rate than small-sized eggs. On both total egg set and fertile egg set, weight of big eggs was adversely associated with hatchability. Egg weight was shown to be adversely associated with hatchability on fertile egg set in tiny eggs.

Rajkumar *et al.* (2020) evaluated the Vanaraja female line (PD-2) chicken for growth, production, carcass and egg quality traits and recorded fertility rate of 86.63 per cent, hatchability of 86.72 per cent on fertile egg set, 72.53 per cent on total egg set and with an average shape index of 75.22 and a Haugh unit of 79.0 ± 0.62 .

2.8 EFFECT OF CALCIUM SUPPLEMENTATION ON FEED EFFICIENCY AND TECHNO-ECONOMICS

Houndonougbo (2012) conducted an experiment with supplementing oyster shell and snail shell in 72 ISA-Brown laying hens of 60th to 72nd weeks were assigned in to four dietary groups, 18 hens in nine cages and two hens each.

The extra supplementation of oyster shell and snail shell increased the cost of feeding and the feed efficiency was not economically beneficial. The economic feed efficiency calculated was significantly not beneficial in snail shell supplemented group and values were 2.12, 2.19, 2.28 and 2.18 in four groups (O, OS, S, and S+).

Padhi *et al.* (2014) studied the techno-economics of exotic crossbreds having multicolour plumage reared under backyard poultry farming and reported that up 72 weeks of age, each bird produced a total of 148 eggs. At 40 weeks, the egg weight was 50.70 g.

Karuna *et al.* (2017) studied the growth and production performance of Vanaraja, Gramapriya and Aseel varieties of 1500 birds were supplied to rural and tribal families in West Godavari District of Andhra Pradesh and reported that the performance of Vanaraja and Gramapriya under tropical condition of the West Godavari were well adapted to local agro-climatic condition under free range system of production. The total gross income per hen/year Rs. 1475.2, Rs. 1468.00 and Rs. 871.00, respectively among Vanaraja, Gramapriya and Aseel varieties.

Shekhar and Ranjan (2020) studied the performance of 920 Vanaraja birds in terms of mean body weight, mean egg production, egg weight and mortality under backyard farming in Koderma district of Jharkhand, India. The total cost of production per farm family (10 birds) was Rs. 3747.50. The total gross income earned from sale of eggs and birds for Vanaraja birds were Rs. 11470.00 and the net income was Rs. 7722.50. The benefit cost ratio (B: C) was recorded 1:3.06.

3. MATERIALS AND METHODS

The materials used and methodology adopted during the conduct of research work is presented in this chapter.

3.1 STUDY MATERIAL COLLECTION

About 150 kg of egg shells were collected from bakeries, washed in tap water and soaked and then sundried for 48 hours. The shell membrane was manually removed and, packed in bags for further processing. Before grinding, the sundried egg shell was dried again in hot air oven at 120° C for 24 hours and powdered in laboratory mill. The shell grit was purchased from the local market and processed.

3.1.1 Calculation of particle sizes distribution of egg shell powder and shell grit

About, 100 g of representative samples of egg shell and shell grit were manually sieved using 0.75 (710 micron) and 2.00-mm (2057 micron) BSS 8 and BSS 22 sieves, respectively for five minutes at one minute interval. The particles which were not passed through BSS 8 and BSS 22 sieves were weighed in electronic analytical balance and particles passed the BSS 8 sieve were also weighed and calculated in per cent according to Wang *et al.* (2014).

3.1.2 *In-vitro* solubility of egg shell and shell grit

The solubility of calcium sources was determined by *in-vitro* limestone solubility method proposed by Zhang & Coon, (1997). Two grams of representative samples of egg shell and shell grit used in the experiment was put into 200 ml 0.2 N hydrochloric acid solution, warmed to 42 °C for 15 min in a water bath with slow stirring using glass rod at regular interval. After 10 min reaction time, the solution was filtered by using pre-weighed ashless Whatman Filter paper No:1 and weighed after drying in hot air oven at 60 °C for 20 h.

The solubility was calculated as mentioned below

$$\text{Solubility (per cent)} = 1 - \left\{ \frac{\text{weight of un-dissolved material (g)}}{\text{weight of the sample (g)}} \right\} \times 100$$

3.2 EXPERIMENTAL SHED

3.2.1 Disinfection

The experimental pens and cages were properly cleaned before the housing of the birds. The feeders, waterers and other equipment were cleaned and disinfected with KohrsolinTh Liquid with a dilution of 5 ml/ L of water and sun dried. The disinfection of the shed was done by spraying same disinfectant with dilution of 10 ml/ L of water.

3.2.2 Housing

In experiment-I, male birds were reared in experimental cages during the whole experiment period. In experiment-II, birds were reared under deep litter system during the whole experiment period. Wood shavings were spread to a thickness of 5 cm in each pen. Each replicate was housed in separate pen in the same shed with facilities for feeding and watering. All the birds were maintained under standard management conditions prevailing in the farm during the entire period of experiment. The birds were provided with two times feed and water at all times. They were exposed to a 16 hours photoperiod (16 Light: 8 Dark) daily and lights were turned off manually.

3.3 EXPERIMENTAL DESIGN, RATION AND FEEDING TRIAL

The experiments were conducted by using the facilities available at Poultry Farm, Instructional Livestock Farm Complex (ILFC), Departments of Animal Nutrition, Animal Reproduction and Gynaecology, and Veterinary Biochemistry, College of Veterinary and Animal Sciences (CVAS), Pookode.

3.3.1 Experimental design

The Gramasree parent stock of roosters and laying hens housed in cages and deep litter poultry pens in Poultry Farm, ILFC was selected to conduct the feeding experiment.

In experiment-I, eighteen Gramasree roosters of 32 weeks of age were selected for semen quality studies and roosters were selected based on their body weight, The selected roosters were randomly assigned to three experimental groups *viz.*, G1, G2 and G3 with six birds (above 2 kg body weight) in each group and separately housed in male cages. In experiment II, three hundred sixty Gramasree breeder hens and 36 roosters (Gramasree) of 32 weeks of age were randomly allotted to G4 and G5 experimental groups with three replicates and 60 birds of female and six birds of male in each replicate and housed in deep litter system. The experiments I and II were conducted at same period during March'21 to May'21 for a period of 91 days.

3.3.2 Experimental Ration

The experimental ration was prepared at Revolving Fund, Feed Mill, College of Veterinary and Animal Sciences, Mannuthy. The breeder diet (basal diet) for laying hens was prepared as per ICAR (2013) nutrient requirements and same basal diet was fed to roosters housed in cages and deep litter pens. After feeding, the weighed quantity of egg shell powder (above 2 mm particle size) and shell grit (above 2 mm particle size) was given as free choice supplementation in the morning and evening as mentioned in the Table-1.

3.3.3 Feed and calcium intake

The feed, egg shell powder and shell grit offered quantity and left over were weighed on daily basis in experiment-I and II birds during 91 experimental days. The calcium intake from feed and different calcium sources were calculated at weekly interval.

Table 1. Ingredient composition of Gramasree layer ration

Raw material	Ingredient composition (%)
Maize	54.0
De-oiled Rice Bran	5.0
Wheat Bran	4.0
Soya bean meal	25.40
Limestone powder	4.0
Shell grit	5.0
Di Calcium Phosphate	1.40
DL-Methionine (98.5 %)	0.20
Salt	0.50
Trace Mineral Mix	0.125
Vitamin Premix	0.05
Choline Cholride	0.20
Toxin Binder	0.10
Liver extract powder	0.025
Total	100

Table 2. Experimental design

Groups	Experiment-I
G1	Layer breeder feed (basal diet) to roosters in cages
G2	Layer breeder feed (basal diet) + egg shell powder (above 2 mm particle size) to roosters in cages
G3	Layer breeder feed (basal diet) + shell grit (above 2 mm particle size) to roosters in cages
Experiment-II	
G4	Layer breeder feed (basal diet) to roosters and layers + egg shell powder in deep litter.
G5	Layer breeder feed (basal diet) to roosters and layers + shell grit in deep litter.

3.4 SEMEN QUALITY

At weekly interval, the semen was collected in a sterile glass funnel by abdominal massage method (Burrows and Quinn, 1937) from four roosters housed in cages from each group from 33rd to 44th weeks of age. The semen collection by abdominal massage method was depicted in Fig 1. The semen diluent was prepared by dissolving 0.8 g of NaCl, 1.374 g of N-Tris methyl, 2-amino Ethane Sulphonic acid (TES), 2.75 ml of 1 molar NaOH and 0.6 g of glucose in 100 ml of double distilled water as per Chaudhuri and Lake (1988). Immediately after semen collection, the semen was four-fold diluted with semen diluent and stored for further semen quality analysis.

3.4.1 Semen volume (ml)

A tuberculin syringe of 1.0 ml capacity, graduated to measure 0.01 ml was used to quantify the ejaculated semen volume before dilution, separately for each rooster as described by Shattner and Andrews (1948).

3.4.2 Semen colour (Grade)

The semen colour was scored 1–5 by visual examination scoring pattern described by McDaniel and Craig (1959).

Score 1	Watery or clear semen
Score 2	Watery semen with white streaks
Score 3	Medium white semen
Score 4	Thick white semen
Score 5	Very viscous chalky white semen

3.4.3 Sperm motility (per cent)

A drop of diluted semen was taken from receptacle with the help of a Pasteur pipette on a dry, clean glass slide and was spread uniformly by keeping a cover slide. This slide was examined quickly under the power of light microscope (40x). On the basis of the activity of swirls, semen was graded and thus motility of spermatozoa was scored in per cent as suggested by Wheeler and Andrews (1943).

3.4.4 Sperm concentration (billion/ml)

Sperm concentration was estimated as per the procedure suggested by Taneja and Gowe (1960). A sample of well mixed diluted semen was drawn up to the 0.5 mark of the RBC pipette. The pipette was filled to 101 mark using formal saline (saline with few drops of formalin) as diluting fluid and agitated sufficiently to mix the contents. A thick cover glass was placed over the well cleaned counting chamber of Neubauer Hemocytometer. A drop of diluted semen from RBC pipette was charged to the counting chamber. The sperm cells were allowed to settle for five minutes, chamber was focused under high power objective and a total of five large squares each consisting of 16 small squares was focused to count the number of sperms. On each square, all the sperms in the centre and those touching the upper and right-hand borders were counted and those straddling the other two edges were ignored. The sperm concentration is calculated by the formula,

$$\text{Sperm concentration (billion/ml)} = \frac{N \times D \times 4000 \times 1000}{n}$$

Where,

N = Number of spermatozoa counted

D = Dilution rate

n = Number of tertiary squares counted (here 80)

3.5 EGG PRODUCTION

The eggs were collected morning and afternoon from each group and recorded on daily basis. The hen-day and hen-housed egg production was calculated using the following formulae. (Xu *et al.*, 2006; Bonekamp *et al.*, 2010; Lee *et al.*, 2021).

3.5.1 Hen-Day Egg Production (HDEP)

Hen-day egg production is the percentage of total number of eggs laid by the flock in a day divided by the number of hens alive on that day.

$$\text{HDEP (\%)} = \frac{\text{Total number of eggs produced on a day}}{\text{Total number of hens present on that day}} \times 100$$

3.5.2 Hen-Housed Egg Production (HHEP)

Hen-Housed Egg Production is the percentage of total number of eggs produced during a day by the number of birds in the flock at the beginning of the period.

$$\text{HHEP (\%)} = \frac{\text{Total number of eggs laid on a day}}{\text{Total number of hens housed at the beginning of laying period}} \times 100$$

3.5.3 Egg Mass

$$\text{Average egg mass (g/hen/day)} = \text{HDEP (\%)} \times \text{Average egg weight (g)}$$

3.5.4 Feed Conversion Ratio (per kg egg mass)

$$\text{FCR (per kg egg mass)} = \frac{\text{Kg of feed consumed}}{\text{Kg of egg produced}}$$

3.5.5 Feed Conversion Ratio (per dozen eggs)

$$\text{FCR (per dozen eggs)} = \frac{\text{Kg of feed consumed} \times 12}{\text{Total eggs produced}}$$

3.6 EGG QUALITY CHARACTERISTICS

Totally, six eggs were randomly collected from each replicate in a group in a week on alternate days and studied for both internal and external egg quality (Rath *et al.*, 2015).

Figure-1: Experimental-I: Semen Collection by abdominal massage method



Figure-2: Egg length measured with the help of vernier calipers



Figure-3: Egg width measured with the help of vernier calipers



Figure-4: Measuring the Specific gravity of egg by floatation technique



Figure-5: Egg broken open at the middle with the help of a blunt end of a knife



The measuring methods of egg length, width, specific gravity, broken egg, albumen diameter, yolk diameter, albumen height, yolk colour and shell thickness were depicted in Fig. 2, 3, 4, 5, 6, 7, 8, 9 and 10, respectively.

3.6.1 External egg quality characteristics

3.6.1.1 Egg Weight (g)

Individual egg weight was recorded during the entire experimental period using 0.01g sensitive electronic balance.

3.6.1.2 Egg Length (mm)

The egg length which is also referred to as the long border of an egg was measured with the help of vernier calipers with minimum unit reading up to 0.01mm.

3.6.1.3 Egg Width (mm)

The egg width which is also referred to as the shorter portion of an egg was measured with vernier calipers with minimum unit reading up to 0.01mm.

3.6.1.4 Shape Index (Per cent)

The length and width of an egg were measured with vernier calipers with minimum unit reading up to 0.01 mm.

3.6.1.5 Specific Gravity

Specific gravity of individual egg was determined in different salt solutions with different density prepared at 0.01 increments from 1.060 to 1.110, by floatation technique of Bernier (1955). The eggs were immersed in water first before place it in different salt solutions to avoid absorption of salt water. The specific gravity was noted, when the egg was floated in particular concentration of salt solution. The eggs were first immersed in salt solution having lowest concentration.

3.6.2 Internal egg quality characteristics

3.6.2.1 Albumen Height (mm)

The egg was broken at the middle with the help of a blunt end of a knife. The egg content was poured on a petri-dish placed on a perfectly leveled glass table. Height of the thick albumen was measured with the help of spherometer.

3.6.2.2 Yolk Colour (Grade)

The yolk colour was measured by using yolk fan and color shade compared with egg yolk was depicted in Fig.10 according to Nolan *et al.* (2016).

3.6.2.3 Yolk Height (mm)

The yolk height was measured after separating the yolk from albumen with the help of spherometer.

3.6.2.4 Yolk Index (Per cent)

The yolk index, which is an indirect measurement of the spherical shape of the yolk and strength of the yolk membrane, was calculated from yolk height and yolk diameter of the egg.

$$\text{Yolk Index (Per cent)} = \frac{\text{Yolk Height (mm)}}{\text{Mean Yolk diameter (mm)}} \times 100$$

3.6.2.5 Yolk Weight (g)

The yolk was carefully separated from the albumen, then adherent albumen was removed by rolling the yolk over a filter paper and yolk weight was recorded.

3.6.2.6 Haugh Unit Score

Haugh unit was determined by the following formula,

$$\text{HU} = 100 \log (H - 1.7 W^{0.37} + 7.56)$$

Where, H = height of the albumen (mm) and W = egg weight (g)

Figure-6: Measuring the thick albumen diameter with vernier

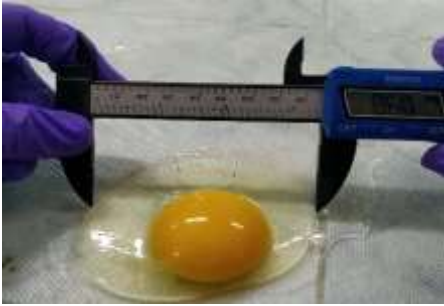


Figure-7: Measuring the yolk diameter with vernier caliper



Figure-8: Measuring the thick albumen height with spherometer



Figure-9: Measuring the shell thickness with the help of screw gauge



Figure-10: Measuring the yolk colour with yolk fan



3.6.3 Egg Shell Quality

3.6.3.1 Egg Surface Area

The egg surface area of an individual egg was calculated using the formula of Carter (1975).

$$\text{E.S.A (cm}^2\text{)} = 3.9782W^{0.7056}$$

Where,

W is the weight of the egg in grams.

3.6.3.2 Shell Weight (g)

After air drying, the shell weight of an individual egg were calculated using the electronic balance with accuracy of 0.001 g.

3.6.3.3 Shell Per cent

The ratio of egg shell weight (g) to the weight of the whole egg was expressed as egg shell per cent.

3.6.3.4 Shell Thickness (mm)

Shell thickness was measured with the help of screw gauge with 0.01 mm precision.

3.6.3.5 Shell Weight per Unit Surface Area

Shell weight per unit surface area was calculated by dividing the shell weight with egg surface area of the given egg.

$$\text{Shell weight per surface area (mg/cm}^2\text{)} = \frac{\text{Shell weight (mg)}}{\text{Egg surface area (cm}^2\text{)}}$$

3.6.3.6 Breaking Strength

The breaking strength of individual eggs were calculated using the formula of Arad and Marder (1982).

$$\text{Breaking Strength (g)} = 50.86 \times W^{0.915}$$

Where,

W is the weight of egg in grams.

3.7 TIBIAL ASH AND CALCIUM

At end of experimental period, four roosters housed in cages and four layers in deep litter were randomly selected to estimate tibial ash and calcium content (Gongruttananun, 2011c).

3.8 BLOOD ANALYSIS

Two ml of blood was collected from the wing vein of each bird using 22-gauge 3 ml syringe and transferred into a clot activator tube. The serum was separated after centrifugation at 3000 rpm for 10 minutes.

The serum Calcium and Phosphorus (direct method, Farrell E C, 1984) were measured using a Semi Automated Clinical Chemistry Analyser (Merck, Microlab 300). The standard biochemical kits used for serum calcium and phosphorus assays were purchased from Monlab Test and Spinreact, respectively.

3.8.1 Serum Calcium

The serum calcium level was analysed in samples as per o-Cresolphtalein principle following Farrell E. C. (1984) method. The standard biochemical kit was purchased from MONLAB, SL, Barcelona and samples were analysed in semi-automated clinical chemistry analyser (Merck, Microlab 300) at 570 nm.

Laboratory procedure

The working reagent was prepared by mixing reagent 1 (R1) and reagent 2 (R2) at 1:1 ratio. Before starting the analysis, the instrument was standardized by using blank (2000 μL of working reagent) and standard solution (2000 μL of working reagent + 20 μL of standard, incubated for 5 min.) at 570 nm. The 20 μL experimental serum sample was mixed 2000 μL of working reagent at incubated for 5 minutes at room temperature. The absorbance of sample was read at 570 nm against standard. The serum calcium content was expressed in mg/dl.

3.8.2 Serum Phosphorus

Inorganic phosphate reacts in acid medium with ammonium molybdate to form a phosphomolybdate complex with yellow color. The intensity of the color formed is proportional to the inorganic phosphorus concentration in the sample

The blank was prepared with 1000 μL of working reagent, standard with 1000 μL of working reagent and 10 μL of standard solution and sample with 1000 μL of working reagent and 10 μL of serum. They were incubated for 5 minutes at room temperature. The absorbance of standard and sample were read against reagent blank at 340 nm. The calcium content was measured using Semi Automated Clinical Chemistry Analyser.

3.9 FERTILITY AND HATCHABILITY

The fertility and hatchability percentage of the eggs laid by the experimental birds during 33rd to 44th weeks of age were calculated from number of fertile eggs counted on 18th day, after setting in the setter by using candling equipment and number of chicks hatched out on 21st day of incubation, respectively. Totally, 180 eggs were collected from each replicate from G4 and G5 group birds in a week were separately labelled to calculate the fertility and hatchability percentage. The eggs were hatched out hatching facilities at Poultry Farm, ILFC, College of Veterinary and Animal Sciences, Pookode.

The fertility per cent was calculated for every setting (Patra *et al.*, 2016).

$$\text{Fertility (per cent)} = \frac{\text{Number of fertile eggs}}{\text{Total number of setting eggs}} \times 100$$

The hatchability per cent was calculated for every hatch (Patra *et al.*, 2016).

$$\text{Hatchability (per cent)} = \frac{\text{Number of chicks}}{\text{Total number of setting eggs}} \times 100$$

3.10 CHEMICAL COMPOSITION

The chemical composition of the layer breeder feed, egg shell powder and shell grit were analysed as per AOAC (2016).

The morphological, topographical (images) and elemental composition of egg shell powder and shell grit were studied using scanning electron microscope with Energy Dispersive Spectrometer (JEOL-JSM-IT 200 with EDS) at Annamalai University, Tamil Nadu.

3.11 TECHNO-ECONOMICS OF PRODUCTION

The cost of feeding supplemental calcium sources as free choice for each treatment was calculated based on the purchased and process cost of egg shell powder and shell grit.

3.12 STATISTICAL ANALYSIS

Data collected on various parameters were subjected to statistical analysis using SPSS version 21.0 using One Way ANOVA method as per described by Snedecor and Cochran (1994).

4. RESULTS

The results obtained from feeding trials with respect to the effect of feeding different calcium sources in Gramasree breeder diet are presented in this chapter.

4.1 CHEMICAL COMPOSITION OF RATION FED TO GRAMASREE BIRDS

The chemical composition of layer breeder ration of laying hens and roosters in experiment I and II are presented in the Table-3 (dry matter basis).

Table 3. Chemical composition of ration fed to experimental birds during 32nd to 44th weeks of age

Attributes (%)	Layer breeder feed (basal diet) (%)	Eggshell (%)	Shell grit (%)
Moisture	10.70 ± 0.14	0.56 ± 0.24	0.70 ± 0.24
Crude Protein	17.65 ± 0.24	8.30 ± 0.41	7.47 ± 0.41
Crude Fibre	9.26 ± 0.15	2.90 ± 0.26	1.58 ± 0.26
Ether Extract	2.29 ± 0.04	0.70 ± 0.06	0.48 ± 0.06
Total Ash	14.99 ± 0.35	93.45 ± 0.60	94.86 ± 0.60
Acid insoluble ash	0.71 ± 0.07	0.34 ± 0.11	0.97 ± 0.11
Calcium	4.85 ± 0.27	42.16 ± 0.48	39.11 ± 0.48
Phosphorous	1.90 ± 0.19	1.70 ± 0.32	3.46 ± 0.32

4.2 PARTICLE SIZE AND *IN-VITRO* SOLUBILITY OF CALCIUM SOURCES IN THE EXPERIMENT

The shell grit and egg shell used as calcium sources in the experiment were analysed for its particle size distribution and solubility and results are presented in table-4.

Table 4. Particle size and *In-vitro* solubility of shell grit and egg shell supplemented as free choice feeding during experimental period

Particle size (mm)	Egg shell	Shell grit	SEM	P value
Quantity (% , by weight)				
Coarse (> 2.00)	84.13 ^a	69.1 ^b	0.46	0.00*
Medium (< 2.00)	15.36 ^b	22.56 ^a	0.43	0.00*
Fine (< 0.75)	0.39 ^b	7.01 ^a	0.06	0.00*
<i>In-vitro</i> solubility (%)				
Coarse (> 2.00)	40.6 ^b	56.21 ^a	0.54	0.00*
Medium (< 2.00)	57.33 ^b	76.51 ^a	1.35	0.00*
Fine (< 0.75)	55.39 ^b	92.44 ^a	0.74	0.00*

^{a, b} Mean values with different superscripts within a row differ significantly

* Significant at $p < 0.01$

4.3 SCANNING ELECTRON MICROSCOPE OF EGG SHELL AND SHELL GRIT WITH ENERGY DISPERSIVE SPECTROMETER (EDS)

The elemental composition of egg shell and shell grit were presented in Table-5 and Table-6, respectively. The egg shell and shell grit used in this study contains 94.25 ± 0.64 and 96.06 ± 0.50 per cent of calcium on the surface, respectively. The scanning electron microscopic image of egg shell and shell grit used is presented in the figure-11 and figure-12.

Table 5. Element composition on the surface of egg shell

Element	Line	Mass (%)	Atom (%)
Mg	K	1.72 ± 0.11	2.78 ± 0.18
S	K	4.03 ± 0.13	4.93 ± 0.16
Ca	K	94.25 ± 0.64	92.28 ± 0.63

Table 6. Element composition on the surface of shell grit

Element	Line	Mass (%)	Atom (%)
Al	K	3.94 ± 0.12	5.74 ± 0.17
Ca	K	96.06 ± 0.50	94.26 ± 0.49

Figure No.11: Scanning Electron Microscopic image of egg shell with Energy Dispersive Spectrometer (EDS)

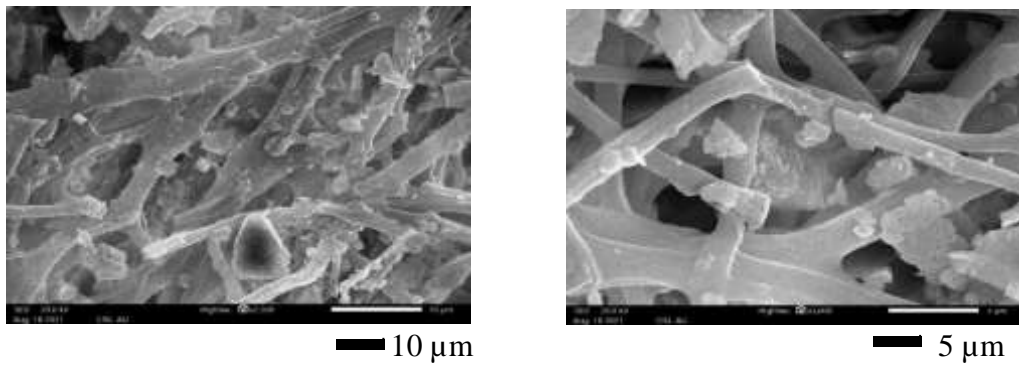
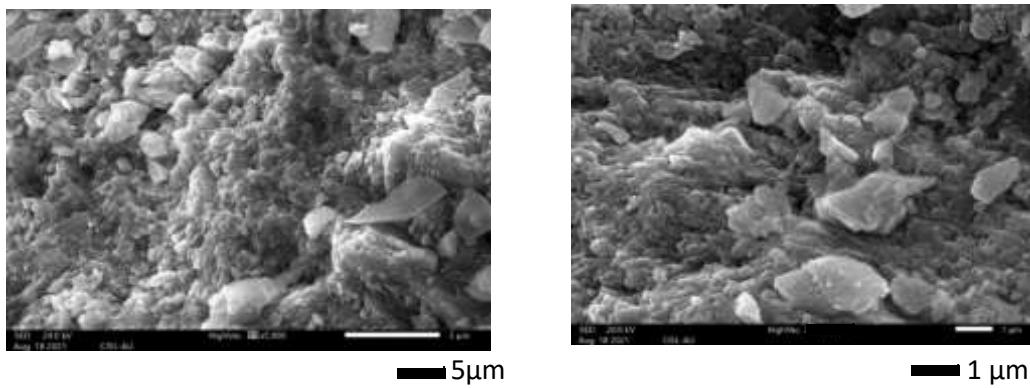


Figure No. 12: Scanning Electron Microscopic image of shell grit with Energy Dispersive Spectrometer (EDS)



4.4 EFFECT OF CALCIUM SUPPLEMENTATION IN MALE BIRDS

The effect of feeding eggshell and shell grit to roosters are studied daily feed intake, calcium intake, semen parameters are presented in the Table-7, Table-8, Table-9 and Table-10, respectively.

4.4.1 Effect of calcium supplementation on feed intake, calcium intake and body weight of roosters in cage

The recorded values on daily feed, eggshell and shell grit intake of Gramasree roosters housed in cages from 33 to 44 weeks of age for three dietary groups are presented in the Table-7. The grand mean feed intake of the three groups is 87.58, 86.86 and 88.28 g/rooster/day, respectively.

The calculated total calcium intake and body weight of Gramasree roosters recorded at different age during experimental period from 32 to 44 weeks of age in cage system for three dietary groups are presented in the Table-8. The grand mean of total calcium intake are 3.46, 3.49 and 3.25 g/rooster/day respectively among the three groups. The body weight of the bird at 32 and 44 weeks of age are presented in the Table-12.

4.4.2 Effect of calcium supplementation on semen quality parameters

The mean values of semen volume and colour of Gramasree roosters recorded at different age during experimental period from 33 to 44 weeks of age in cage system for three dietary groups are presented in the Table-9. The grand mean of semen volume of G1, G2 and G3 are 0.13, 0.16 and 0.14 ml/rooster, respectively and the grand mean values of semen colour are 2.35, 2.80 and 2.83.

The mean sperm motility with grand mean of 47.92, 61.04 and 61.46 per cent, respectively among G1, G2 and G3 and sperm concentration with grand mean of 4.25, 5.49 and 5.31 billion per ml, respectively for G1, G2 and G3 from 33 to 44 weeks and presented in the Table-10.

Table 7. Mean daily feed, eggshell and shell grit intake of Gramasree roosters housed in cages (g/rooster/day)

Feed intake of Gramasree roosters													
Age (weeks)	32	33	34	35	36	37	38	39	40	41	42	43	44
G1	86.00 ^b	73.67 ^b	67.33 ^c	74.33 ^c	59.33 ^c	64.67 ^c	69.00 ^c	85.00 ^b	108.00 ^a	120.00 ^a	106.67 ^a	108.00 ^a	115.00 ^a
G2	89.33 ^b	84.67 ^a	81.33 ^b	94.67 ^a	76.33 ^a	79.00 ^a	76.00 ^a	82.00 ^c	91.00 ^c	100.67 ^c	95.67 ^b	87.33 ^c	93.67 ^c
G3	94.67 ^a	84.67 ^a	85.00 ^a	85.67 ^b	74.33 ^b	73.00 ^b	71.00 ^b	91.00 ^a	106.67 ^b	104.00 ^b	93.00 ^c	96.33 ^b	94.67 ^b
SEM	0.60	0.19	0.16	0.19	0.90	0.11	0.10	0.33	0.11	0.11	0.16	0.16	0.16
p-value	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Eggshell (G2) and shell grit (G3) intake (g) of Gramasree roosters													
G2	0.59 ^a	0.50 ^b	0.76 ^b	0.85 ^a	0.40 ^a	0.28 ^a	0.36 ^a	0.50 ^b	1.13 ^a	1.46 ^a	0.86 ^a	0.60 ^b	0.43 ^b
G3	0.50 ^b	0.62 ^a	1.14 ^a	0.64 ^b	0.31 ^b	0.22 ^b	0.33 ^b	0.86 ^a	0.93 ^b	1.43 ^b	0.79 ^b	0.95 ^a	0.67 ^a
SEM	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.00
p-value	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.01*	0.00*	0.00*	0.01*	0.00*	0.00*	0.00*

a, b and c Mean values with different superscripts within a row differ significantly

*Significant at p<0.01

Table 8. Mean daily calcium intake of Gramasree roosters from feed (G1), egg shell (G2) and shell grit (G3) and total calcium intake housed in cage (g/rooster/day)

Calcium intake of Gramasree roosters from feed													
	Age (Weeks)												
Groups	32	33	34	35	36	37	38	39	40	41	42	43	44
G1	3.15 ^c	2.69 ^c	2.46 ^c	2.71 ^c	2.16 ^c	2.37 ^c	2.52 ^c	3.1 ^b	3.95 ^a	4.38 ^a	3.90 ^a	3.95 ^a	4.21 ^a
G2	3.27 ^b	3.10 ^b	2.98 ^b	3.46 ^a	2.79 ^a	2.89 ^a	2.79 ^a	2.99 ^c	3.32 ^c	3.67 ^c	3.50 ^b	3.20 ^c	3.42 ^c
G3	3.46 ^a	3.11 ^a	3.11 ^a	3.15 ^b	2.73 ^b	2.66 ^b	2.59 ^b	3.33 ^a	3.90 ^b	3.80 ^b	3.40 ^c	3.53 ^b	3.46 ^b
SEM	0.04	0.01	0.01	0.01	0.05	0.01	0.00	0.02	0.01	0.01	0.00	0.00	0.00
p-value	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}
Calcium intake of Gramasree roosters from eggshell (G2) and shell grit (G3)													
G2	0.23 ^a	0.20 ^b	0.30 ^b	0.33 ^a	0.16 ^a	0.11 ^a	0.14 ^a	0.19 ^b	0.44 ^a	0.57 ^b	0.34	0.24 ^b	0.17 ^b
G3	0.21 ^b	0.26 ^a	0.48 ^a	0.27 ^b	0.13 ^b	0.09 ^b	0.12 ^b	0.36 ^a	0.39 ^b	0.60 ^a	0.33	0.40 ^a	0.28 ^a
SEM	0.002	0.003	0.001	0.002	0.001	0.004	0.001	0.002	0.002	0.003	0.003	0.002	0.002
p value	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.03 [*]	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.52 ^{ns}	0.00 ^{**}	0.00 ^{**}
Total Calcium intake of Gramasree roosters from feed (G1), eggshell (G2) and shell grit (G3)													
G1	3.15 ^c	2.69 ^c	2.46 ^c	2.71 ^c	2.16 ^c	2.37 ^c	2.52 ^c	3.10 ^c	3.95 ^b	4.38 ^b	3.90 ^a	3.95 ^a	4.21 ^a
G2	3.48 ^b	3.36 ^b	3.46 ^b	3.73 ^a	2.92 ^a	2.98 ^a	2.93 ^a	3.35 ^b	3.71 ^c	4.28 ^c	3.83 ^b	3.60 ^c	3.71 ^c
G3	3.67 ^a	3.37 ^a	3.59 ^a	3.42 ^b	2.86 ^b	2.75 ^b	2.73 ^b	3.69 ^a	4.29 ^a	4.40 ^a	3.73 ^c	3.93 ^b	3.74 ^b
SEM	0.04	0.01	0.01	0.01	0.05	0.01	0.01	0.02	0.01	0.01	0.01	0.00	0.01
p-value	0.214 ^{ns}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.247 ^{ns}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}

^{a, b, c} Mean values with different superscripts within a row differ significantly

^{**} Significant at p<0.01 ^{*} Significant at p<0.05 ^{ns} non-significant

Table 9. Mean semen volume and colour of Gramasree roosters at different weeks

Age (week)	Semen volume (ml/rooster/collection)					Semen colour (grade)				
	Groups			SEM	p-value	Groups			SEM	p-value
	G1	G2	G3			G1	G2	G3		
33	0.15	0.12	0.14	0.02	0.388 ^{ns}	2.00	2.75	2.75	0.31	0.201 ^{ns}
34	0.12 ^a	0.12 ^a	0.04 ^b	0.02	0.007 ^{**}	2.75	2.50	2.50	0.36	0.856 ^{ns}
35	0.06	0.1	0.07	0.03	0.619 ^{ns}	1.50	2.75	2.25	0.35	0.091 ^{ns}
36	0.13	0.12	0.20	0.06	0.465 ^{ns}	1.50 ^a	2.75 ^{ab}	3.00 ^b	0.32	0.020 [*]
37	0.10	0.17	0.20	0.05	0.406 ^{ns}	2.75	3.00	3.00	0.28	0.767 ^{ns}
38	0.15	0.18	0.13	0.05	0.735 ^{ns}	2.75	3.00	3.25	0.20	0.274 ^{ns}
39	0.17	0.16	0.19	0.06	0.954 ^{ns}	2.75	3.00	2.75	0.20	0.622 ^{ns}
40	0.14	0.17	0.14	0.05	0.908 ^{ns}	2.25	3.00	2.75	0.20	0.075 ^{ns}
41	0.15	0.19	0.15	0.04	0.728 ^{ns}	2.25	2.5	3.00	0.22	0.100 ^{ns}
42	0.15	0.20	0.10	0.03	0.170 ^{ns}	2.50	3.00	2.75	0.22	0.323 ^{ns}
43	0.12	0.23	0.15	0.04	0.223 ^{ns}	3.00	2.75	3.00	0.14	0.405 ^{ns}
44	0.16	0.14	0.16	0.03	0.845 ^{ns}	2.25	2.75	3.00	0.20	0.075 ^{ns}

^{a, b} Mean values with different superscripts within a row differ significantly

^{**} Significant at p<0.01

^{*} Significant at p<0.05

^{ns} non-significant

Table 10. Mean sperm motility and sperm concentration (billion/ml) of Gramasree roosters at different weeks

Age (week)	Sperm motility (%)					Sperm Concentration (billions/ml)				
	Groups			SEM	p- value	Groups			SEM	p- value
	G1	G2	G3			G1	G2	G3		
33	62.50	55.00	50.00	10.77	0.72 ^{ns}	4.79	5.23	5.65	0.67	0.67 ^{ns}
34	65.00	50.00	52.50	9.39	0.51 ^{ns}	4.86	4.17	5.00	0.83	0.76 ^{ns}
35	40.00	57.50	50.00	7.22	0.28 ^{ns}	3.25	5.62	5.26	1.01	0.25 ^{ns}
36	40.00 ^b	72.50 ^a	62.50 ^{ab}	7.36	0.03*	3.53	5.82	4.88	1.07	0.35 ^{ns}
37	45.00	67.50	75.00	7.95	0.06 ^{ns}	3.67	5.11	6.11	0.68	0.09 ^{ns}
38	50.00	65.00	62.50	9.68	0.53 ^{ns}	4.24	5.33	4.90	0.79	0.63 ^{ns}
39	40.00 ^b	55.00 ^{ab}	72.50 ^a	6.61	0.02*	4.40	5.71	6.20	1.06	0.49 ^{ns}
40	52.50	65.00	45.00	8.94	0.32 ^{ns}	4.88	5.69	5.85	1.03	0.78 ^{ns}
41	40.00	52.50	60.00	8.62	0.30 ^{ns}	3.86	6.43	6.11	0.99	0.19 ^{ns}
42	45.00	55.00	62.50	7.95	0.34 ^{ns}	4.15	5.87	4.09	0.87	0.31 ^{ns}
43	42.50 ^b	65.00 ^a	80.00 ^a	5.20	0.00**	4.80	6.15	6.12	0.86	0.48 ^{ns}
44	52.50	72.50	65.00	8.58	0.30 ^{ns}	4.62	4.79	3.53	0.65	0.37 ^{ns}

a, b and c Mean values with different superscripts within a row differ significantly

** Significant at p<0.01

* Significant at p<0.05

^{ns} non-significant

Table 11. Mean serum calcium and phosphorus of Gramasree roosters housed in cage (mg/dL)

Age (week)	Calcium (mg/dL)					Phosphorus (mg/dL)				
	Group			SEM	p-value	Group			SEM	P-value
	G1	G2	G3			G1	G2	G3		
33	11.30	10.67	11.00	0.92	0.88 ^{ns}	7.15 ^a	6.58 ^{ab}	4.33 ^b	0.67	0.05*
34	13.00	12.00	12.67	1.31	0.86 ^{ns}	5.42	6.11	7.47	1.03	0.42 ^{ns}
35	11.00	10.00	11.00	0.82	0.63 ^{ns}	6.26	5.57	7.05	0.86	0.52 ^{ns}
36	11.33	11.00	10.33	1.23	0.85 ^{ns}	8.27 ^a	4.57 ^b	5.66 ^{ab}	0.85	0.05*
37	13.67	12.00	10.00	1.02	0.11 ^{ns}	6.03	7.64	7.59	1.12	0.55 ^{ns}
38	12.67	12.67	11.67	1.56	0.88 ^{ns}	5.53	6.69	4.73	0.88	0.35 ^{ns}
39	12.00	11.00	12.67	1.39	0.71 ^{ns}	5.00	6.98	6.68	0.55	0.09 ^{ns}
40	10.00	11.33	13.33	0.79	0.06 ^{ns}	5.16	5.24	6.70	0.63	0.23 ^{ns}
41	11.67	9.67	13.33	1.29	0.21 ^{ns}	7.87 ^a	6.83 ^a	4.44 ^b	0.35	0.00**
42	10.00	9.67	11.00	1.02	0.65 ^{ns}	7.10	5.79	5.76	1.25	0.71 ^{ns}
43	13.00	12.00	11.33	1.17	0.62 ^{ns}	5.12	4.86	7.60	1.21	0.28 ^{ns}
44	11.67	11.67	10.67	0.75	0.58 ^{ns}	5.37 ^b	8.94 ^a	6.14 ^b	0.77	0.04*

^{a, b} Mean values with different superscripts within a row differ significantly

** Significant at p<0.01

* Significant at p<0.05

^{ns} non-significant

Table 12. Mean values of different parameters in Experiment-I during the experimental period

Parameter	Group			SEM	p-value
	G1	G2	G3		
Feed intake (g)	87.58 ^b	86.86 ^c	88.28 ^a	0.22	0.000*
Egg shell powder (G2) and shell grit (G3) intake (g)	-	0.67 ^b	0.72 ^a	0.00	0.002*
Calcium intake from feed (g/bird/day)	3.20 ^b	3.18 ^c	3.25 ^a	0.01	0.000*
Calcium intake from egg shell and shell grit (g/bird/day)	-	0.26	0.30	0.00	0.061 ^{ns}
Total calcium intake (g/bird/day)	3.20	3.44	3.55	0.01	0.036 ^{ns}
Semen characteristics					
Semen Volume (mL)	0.13	0.16	0.14	0.04	0.54 ^{ns}
Semen Colour (score)	2.35	2.81	2.83	0.25	0.32 ^{ns}
Sperm Motility (%)	47.92	61.04	61.46	8.19	0.28 ^{ns}
Sperm Concentration (x10 ⁹ cells /mL)	4.25	5.49	5.31	0.88	0.45 ^{ns}
Serum parameters					
Calcium (mg/dL)	13.66	17.38	17.43	2.34	0.40 ^{ns}
Phosphorus (mg/dL)	6.19	6.32	6.18	0.85	0.27 ^{ns}
Age	Mean body weight of roosters in cage				
32 weeks (kg)	2.307	2.177	2.400	0.09	0.245
44 weeks (kg)	2.603 ^a	2.311 ^b	2.029 ^c	0.11	0.007*

^{a, b, c} Mean values with different superscripts within a row differ significantly

* Significant at p<0.01

^{ns} non-significant

4.5 EFFECT OF CALCIUM SUPPLEMENTATION IN GRAMASREE BIRDS

4.5.1 Effect of calcium supplementation on feed intake, calcium intake and body weight of Gramasree birds

The recorded values on daily feed, eggshell and shell grit intake of Gramasree birds for two dietary treatments are presented in Table-13. The calculated total calcium intake of Gramasree birds recorded at different weeks for G4 and G5 dietary groups are presented in the Table-14.

Table 13. Mean daily feed and calcium sources intake of egg shell (G4) and shell grit (G5) of Gramasree birds in deep litter (g/bird/day)

Feed intake of Gramasree birds in deep litter													
Age (week)	32	33	34	35	36	37	38	39	40	41	42	43	44
G4	93.00	115.67	121.33	122.67	123.33	126.33	131.67	130.33	129.33	131.00	133.33	134.00	129.98
G5	90.67	114.67	119.67	122.00	122.67	125.33	128.33	128.33	124.00	125.67	129.33	126.33	123.11
SEM	4.68	2.11	2.33	2.05	3.78	3.26	3.43	4.63	5.60	4.42	5.77	6.29	7.03
p-value	0.74 ^{ns}	0.75 ^{ns}	0.64 ^{ns}	0.83 ^{ns}	0.91 ^{ns}	0.84 ^{ns}	0.53 ^{ns}	0.78 ^{ns}	0.54 ^{ns}	0.44 ^{ns}	0.65 ^{ns}	0.44 ^{ns}	0.53 ^{ns}
Calcium sources intake of egg shell (G4) and shell grit (G5) of Gramasree birds in deep litter													
G4	3.36 ^a	3.01	2.96 ^a	3.92 ^a	4.61 ^a	4.97 ^a	5.73 ^a	5.98 ^a	5.85 ^a	6.18 ^a	7.10 ^a	7.06 ^a	7.10 ^a
G5	2.55 ^b	2.47	1.45 ^b	2.10 ^b	2.21 ^b	2.94 ^b	3.63 ^b	3.94 ^b	3.88 ^b	3.83 ^b	4.70 ^b	4.77 ^b	4.38 ^b
SEM	0.16	0.24	0.10	0.09	0.13	0.10	0.15	0.11	0.17	0.16	0.22	0.25	0.23
p-value	0.02*	0.18 ^{ns}	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**

^{a, b} Mean values with different superscripts within a row differ significantly

**Significant at p<0.01 *Significant at p<0.05; ^{ns}non-significant

Table 14. Mean daily total calcium intake of Gramasree birds from feed, egg shell (G4) and shell grit (G5) in deep litter (g/bird)

Calcium intake of Gramasree birds from feed													
Age (week)	32	33	34	35	36	37	38	39	40	41	42	43	44
G4	3.40	4.23	4.43	4.49	4.52	4.62	4.81	4.75	4.73	4.79	4.88	4.90	4.75
G5	3.31	4.20	4.38	4.46	4.48	4.58	4.69	4.69	4.54	4.60	4.73	4.63	4.50
SEM	0.17	0.08	0.08	0.08	0.14	0.12	0.12	0.17	0.20	0.16	0.21	0.23	0.26
p-value	0.72 ^{ns}	0.81 ^{ns}	0.67 ^{ns}	0.77 ^{ns}	0.87 ^{ns}	0.80 ^{ns}	0.53 ^{ns}	0.81 ^{ns}	0.53 ^{ns}	0.44 ^{ns}	0.65 ^{ns}	0.45 ^{ns}	0.53 ^{ns}
Calcium intake of Gramasree birds from eggshell (G4) and shell grit (G5)													
G4	1.32	1.18	1.16 ^a	1.53 ^a	1.81 ^a	1.95 ^a	2.24 ^a	2.34 ^a	2.29 ^a	2.42 ^a	2.78 ^a	2.77 ^a	2.78 ^a
G5	1.08	1.04	0.61 ^b	0.89 ^b	0.94 ^b	1.24 ^b	1.53 ^b	1.66 ^b	1.64 ^b	1.62 ^b	1.99 ^b	2.02 ^b	1.85 ^b
SEM	0.06	0.09	0.04	0.04	0.05	0.04	0.06	0.05	0.07	0.06	0.08	0.10	0.09
p-value	0.06 ^{ns}	0.36 ^{ns}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.01 ^{**}	0.00 ^{**}
Total calcium intake of Gramasree birds from feed, eggshell (G4) and shell grit (G5)													
G4	4.72	5.41	5.59 ^b	6.03 ^b	6.32 ^b	6.57 ^b	7.05 ^b	7.10	7.03	7.22 ^b	7.66	7.66	7.54
G5	4.38	5.24	4.99 ^a	5.34 ^a	5.42 ^a	5.82 ^a	6.22 ^a	6.36	6.18	6.22 ^a	6.72	6.65	6.35
SEM	0.15	0.09	0.09	0.07	0.16	0.15	0.18	0.21	0.24	0.22	0.29	0.32	0.33
p-value	0.20 ^{ns}	0.28 ^{ns}	0.01 ^{**}	0.00 ^{**}	0.02 [*]	0.03 [*]	0.03 [*]	0.07 ^{ns}	0.07 ^{ns}	0.03 [*]	0.08 ^{ns}	0.09 ^{ns}	0.07 ^{ns}

^{a, b, c} Mean values with different superscripts within a row differ significantly

** Significant at p<0.01 * Significant at p<0.05 ^{ns} non-significant

4.6 EFFECT OF CALCIUM SUPPLEMENTATION ON EGG PRODUCTION

4.6.1 Hen-Day and Hen Housed Egg Production of Gramasree layers in deep litter system

The average hen-day egg production of Gramasree layers during experimental period from 33 to 44 weeks were 57.40 per cent in G4 and 54.33 per cent in G5. The mean hen-housed egg production of Gramasree layers during experimental period from 33 to 44 weeks were 53.04 and 53.54 per cent, respectively in G4 and G5.

Table 15. Hen-Day and Hen Housed Egg Production of Gramasree layers during experimental period in deep litter system (Per cent)

Age (week)	Hen-Day Egg Production (%)				Hen-Housed Egg Production (%)			
	Groups		SEM	p-value	Groups		SEM	p-value
	G4	G5			G4	G5		
33	59.13	56.17	4.36	0.66 ^{ns}	50.25	51.63	0.85	0.32 ^{ns}
34	60.62	54.77	3.44	0.30 ^{ns}	53.44	53.61	1.02	0.91 ^{ns}
35	65.27	55.35	4.37	0.18 ^{ns}	53.22	51.78	1.02	0.37 ^{ns}
36	61.22	55.9	4.57	0.46 ^{ns}	54.11	55.06	0.98	0.53 ^{ns}
37	64.39	57.9	4.05	0.32 ^{ns}	53.56	55.33	1.02	0.29 ^{ns}
38	57.65	55.02	3.63	0.64 ^{ns}	53.17	53.39	0.81	0.85 ^{ns}
39	57.09	55.99	3.61	0.84 ^{ns}	53.17	53.73	1.01	0.71 ^{ns}
40	53.53	53.5	1.48	0.99 ^{ns}	53.82	50.98	0.75	0.06 ^{ns}
41	53.86	52.22	3.68	0.77 ^{ns}	51.99	54	0.94	0.21 ^{ns}
42	53.95	52.76	2.65	0.77 ^{ns}	53.71	54.28	0.49	0.46 ^{ns}
43	53.18	52.36	1.72	0.75 ^{ns}	51.96	53.61	1.71	0.53 ^{ns}
44	48.94	49.98	2.89	0.81 ^{ns}	54.11	55.06	0.98	0.53 ^{ns}

^{ns} non-significant

4.6.2 Mean egg mass of Gramasree layers (g/hen/day)

The mean egg mass of Gramasree layers during experimental period for G4 was 30.06 g/hen/day and G5 was 28.87 g/hen/day.

Table 16. Mean egg mass of Gramasree layers from 33 to 44 weeks of age (g/hen/day)

Age (week)	Groups		SEM	p-value
	G4	G5		
33	29.75	29.04	2.51	0.85 ^{ns}
34	32.37	29.26	1.27	0.16 ^{ns}
35	34.74	28.64	2.29	0.13 ^{ns}
36	33.19	30.81	2.85	0.59 ^{ns}
37	34.45	32.02	2.11	0.46 ^{ns}
38	30.66	29.31	1.77	0.62 ^{ns}
39	30.32	30.04	1.69	0.91 ^{ns}
40	28.80	27.29	0.98	0.34 ^{ns}
41	28.04	28.21	2.18	0.96 ^{ns}
42	28.95	28.62	1.17	0.85 ^{ns}
43	27.60	28.04	0.87	0.74 ^{ns}
44	26.48	27.50	1.53	0.66 ^{ns}

^{ns} non-significant

4.6.3 Mean Feed Conversion Ratio per kg egg mass and per dozen eggs of Gramasree birds in deep litter

The calculated mean values of feed conversion ratio (per kg egg mass) from 33 to 44 weeks was 2.84 in G4 and 2.99 in G5 and presented in the Table-17. Similarly, the feed conversion ratio (per dozen eggs) was 2.84 and 2.99, respectively in G4 and G5.

Table 17. Mean Feed Conversion Ratio per kg egg mass and per dozen eggs of Gramasree birds from 33 to 44 weeks of age

Age (week)	Feed Conversion Ratio (per kg egg mass)				Feed Conversion Ratio (per dozen eggs)			
	Groups		SEM	p-value	Groups		SEM	p-value
	G4	G5			G4	G5		
33	2.16	2.21	0.20	0.87 ^{ns}	3.17	3.16	0.32	0.98 ^{ns}
34	2.58	2.87	0.19	0.34 ^{ns}	3.58	3.95	0.18	0.23 ^{ns}
35	2.49	2.95	0.23	0.23 ^{ns}	3.49	4.28	0.35	0.18 ^{ns}
36	2.72	2.93	0.20	0.49 ^{ns}	3.75	4.01	0.31	0.58 ^{ns}
37	2.55	2.85	0.18	0.29 ^{ns}	3.59	3.88	0.26	0.47 ^{ns}
38	2.86	3.00	0.20	0.51 ^{ns}	4.13	4.32	0.27	0.63 ^{ns}
39	3.00	3.02	0.20	0.95 ^{ns}	4.38	4.28	0.28	0.80 ^{ns}
40	3.12	3.16	0.09	0.79 ^{ns}	4.52	4.72	0.23	0.58 ^{ns}
41	3.07	3.17	0.25	0.79 ^{ns}	4.65	4.46	0.40	0.75 ^{ns}
42	3.08	3.15	0.16	0.80 ^{ns}	4.54	4.41	0.21	0.67 ^{ns}
43	3.10	3.22	0.08	0.33 ^{ns}	4.83	4.62	0.18	0.45 ^{ns}
44	3.35	3.28	0.19	0.82 ^{ns}	5.08	4.61	0.23	0.22 ^{ns}

^{ns} non-significant

4.7 EGG QUALITY CHARACTERISTICS

4.7.1 Effect of calcium supplementation on external egg quality parameters

The recorded mean values of egg weight from 33 to 44 weeks was 53.09 g in G4 and 53.59 g in G5 and the shape index was 78.75 and 77.30 in G4 and G5 groups, respectively and the weekly values are presented in the Table-18 and Table-19.

4.7.2 Effect of calcium supplementation on internal egg quality parameters

The recorded and calculated values of internal egg quality of Gramasree layers during experimental period in deep litter system for two dietary groups are presented in Table-20 and Table-21.

Table 18. Mean Egg Weight of Gramasree hens from 33 to 43 weeks of age (g)

Age (week)	Groups		SEM	p-value
	G4	G5		
33	53.44	53.61	1.02	0.91 ^{ns}
34	53.22	51.78	1.02	0.37 ^{ns}
35	54.11	55.06	0.98	0.53 ^{ns}
36	53.56	55.33	1.02	0.29 ^{ns}
37	53.17	53.39	0.81	0.85 ^{ns}
38	53.17	53.73	1.01	0.71 ^{ns}
39	53.82	50.90	0.75	0.06 ^{ns}
40	51.99	54.00	0.94	0.21 ^{ns}
41	53.71	54.28	0.49	0.46 ^{ns}
42	51.96	53.61	1.71	0.53 ^{ns}
43	54.11	55.06	0.98	0.53 ^{ns}
44	53.72	54.18	0.73	0.68 ^{ns}

^{ns} non-significant

Table 19. Mean Egg Length, Breadth and Shape Index of Gramasree hens from 33 to 44 weeks of age

Age (week)	Egg Length (mm)				Egg Width (mm)				Shape Index			
	Groups		SEM	p-value	Groups		SEM	p-value	Groups		SEM	p-value
	G4	G5			G4	G5			G4	G5		
33	55.38	55.36	0.74	0.98 ^{ns}	42.77	42.30	1.01	0.76 ^{ns}	77.81	79.58	1.04	0.30 ^{ns}
34	54.28	54.25	0.70	0.97 ^{ns}	42.85	42.21	0.64	0.52 ^{ns}	78.00	75.67	2.35	0.52 ^{ns}
35	54.94	55.15	0.75	0.86 ^{ns}	47.02	42.70	2.00	0.20 ^{ns}	79.05	73.42	2.57	0.20 ^{ns}
36	54.70	53.82	0.84	0.50 ^{ns}	42.55	43.13	0.97	0.70 ^{ns}	79.18	74.12	1.92	0.14 ^{ns}
37	53.84	55.37	0.69	0.19 ^{ns}	42.09	43.90	0.62	0.10 ^{ns}	81.15	76.99	2.98	0.38 ^{ns}
38	54.34	52.39	2.96	0.67 ^{ns}	42.79	46.65	3.03	0.42 ^{ns}	79.08	78.70	0.48	0.60 ^{ns}
39	54.38	54.17	0.99	0.88 ^{ns}	42.47	41.92	0.72	0.62 ^{ns}	79.50	76.38	0.92	0.07 ^{ns}
40	53.90	53.63	0.96	0.85 ^{ns}	42.60	42.07	0.63	0.58 ^{ns}	78.28	76.99	0.91	0.38 ^{ns}
41	53.60	55.23	0.42	0.05 ^{ns}	42.20	42.40	0.34	0.65 ^{ns}	78.45	78.68	0.34	0.65 ^{ns}
42	53.67	53.93	0.88	0.84 ^{ns}	43.43	42.63	0.74	0.48 ^{ns}	77.08	79.13	0.70	0.11 ^{ns}
43	53.77	54.77	1.50	0.66 ^{ns}	42.47	43.20	0.63	0.46 ^{ns}	78.56	78.29	0.19	0.37 ^{ns}
44	54.07	55.20	0.76	0.35 ^{ns}	42.10	42.00	0.56	0.91 ^{ns}	78.85	79.66	1.06	0.62 ^{ns}

^{ns} non-significant

Table 20. Mean Albumen Height and Haugh Unit of Gramasree hens from 33 to 44 weeks of age

Age (week)	Albumen Height (mm)				Haugh Unit (mm)			
	Groups		SEM	p-value	Groups		SEM	p-value
	G4	G5			G4	G5		
33	6.49	6.96	0.19	0.165 ^{ns}	80.92	84.73	2.05	0.26 ^{ns}
34	7.03	6.99	0.06	0.672 ^{ns}	85.43	85.19	0.57	0.78 ^{ns}
35	7.06 ^b	6.70 ^a	0.06	0.013*	85.40	83.06	0.62	0.06 ^{ns}
36	7.01	6.96	0.05	0.530 ^{ns}	85.15	85.15	0.23	1.00 ^{ns}
37	6.83	6.98	0.15	0.516 ^{ns}	84.27	84.76	0.86	0.71 ^{ns}
38	7.07	7.03	0.19	0.888 ^{ns}	85.60	85.98	1.71	0.88 ^{ns}
39	6.63	6.77	0.31	0.761 ^{ns}	86.75	87.88	1.86	0.69 ^{ns}
40	6.40	6.38	0.16	0.957 ^{ns}	81.48	81.45	1.32	0.99 ^{ns}
41	6.10	6.40	0.31	0.533 ^{ns}	79.58	80.90	2.14	0.69 ^{ns}
42	6.08	6.63	0.26	0.219 ^{ns}	79.40	82.93	1.77	0.23 ^{ns}
43	6.84 ^b	6.00 ^a	0.18	0.030*	84.36 ^b	78.43 ^a	1.26	0.03*
44	6.13	6.62	0.12	0.050 ^{ns}	79.65	73.84	6.23	0.55 ^{ns}

^{a, b} Mean values with different superscripts within a row differ significantly

* Significant at $p < 0.05$ ^{ns} non-significant

Table 21. Mean Yolk Colour, Height (mm) and Yolk Index of Gramasree hens from 33 to 44 weeks of age

Age (week)	Yolk Colour (Yolk colour fan)				Yolk height (mm)				Yolk index			
	Groups		SEM	p-value	Groups		SEM	p-value	Groups		SEM	p-value
	G4	G5			G4	G5			G4	G5		
33	7.33	8.33	0.53	0.25 ^{ns}	15.89	17.89	0.65	0.09 ^{ns}	35.41 ^a	42.65 ^b	1.59	0.03*
34	7.33	7.33	1.27	1.00 ^{ns}	17.39	16.48	0.81	0.47 ^{ns}	40.33	39.19	2.46	0.76 ^{ns}
35	8.33	7.67	0.53	0.42 ^{ns}	16.72	17.01	0.45	0.67 ^{ns}	38.61	40.04	1.45	0.52 ^{ns}
36	7.00	7.67	0.47	0.37 ^{ns}	17.37	16.59	0.53	0.35 ^{ns}	40.83	39.57	0.97	0.41 ^{ns}
37	8.67	7.67	0.33	0.10 ^{ns}	16.42	17.78	0.49	0.12 ^{ns}	38.98	40.94	1.15	0.30 ^{ns}
38	9.00	7.67	0.47	0.12 ^{ns}	16.22	17.59	0.36	0.05 ^{ns}	37.54 ^a	42.01 ^b	1.07	0.04*
39	7.00	7.67	0.47	0.37 ^{ns}	17.60	17.50	0.44	0.90 ^{ns}	41.93	41.16	1.30	0.70 ^{ns}
40	7.33	6.33	0.33	0.10 ^{ns}	16.50	17.16	0.42	0.33 ^{ns}	38.55	40.59	0.76	0.13 ^{ns}
41	7.67	6.67	0.67	0.35 ^{ns}	16.66	16.18	0.24	0.23 ^{ns}	39.02	39.11	0.34	0.86 ^{ns}
42	7.00	7.33	0.47	0.64 ^{ns}	16.86	16.91	0.82	0.97 ^{ns}	39.71	41.06	3.55	0.80 ^{ns}
43	6.67	7.67	0.33	0.10 ^{ns}	17.12	16.50	0.39	0.34 ^{ns}	40.86	38.93	1.26	0.34 ^{ns}
44	8.00 ^b	7.00 ^a	0.00	<0.001**	16.35	16.58	0.39	0.71 ^{ns}	37.89	39.53	1.25	0.41 ^{ns}

^{a, b} Mean values with different superscripts within a row differ significantly

** Significant at p<0.01 * Significant at p<0.05 ^{ns} non-significant

4.7.3 Mean Yolk Weight and Yolk Ratio of Gramasree hens from 33 to 44 weeks of age

The recorded and calculated mean values of yolk weight and yolk ratio of Gramasree hens for two dietary groups are presented in the Table-22.

Table 22. Mean Yolk Weight and Yolk Ratio of Gramasree hens from 33 to 44 weeks of age

Age (week)	Yolk Weight (g)				Yolk per cent (%)			
	Groups		SEM	p-value	Groups		SEM	p-value
	G4	G5			G4	G5		
33	17.75	17.97	1.04	0.89 ^{ns}	31.32	32.60	2.08	0.69 ^{ns}
34	17.33	16.79	0.77	0.65 ^{ns}	31.11	31.00	1.56	0.96 ^{ns}
35	17.75	17.36	0.84	0.76 ^{ns}	32.29	31.44	1.13	0.62 ^{ns}
36	18.69	16.18	0.99	0.15 ^{ns}	34.23	30.05	1.97	0.21 ^{ns}
37	16.04	17.10	0.48	0.20 ^{ns}	29.81	30.88	0.89	0.44 ^{ns}
38	16.79	17.65	0.50	0.29 ^{ns}	30.93	34.23	2.64	0.43 ^{ns}
39	17.08	16.64	0.74	0.70 ^{ns}	40.22	39.64	1.43	0.79 ^{ns}
40	16.18	16.97	1.04	0.62 ^{ns}	30.03	31.59	1.62	0.53 ^{ns}
41	17.99	16.65	0.84	0.32 ^{ns}	33.57	30.13	1.46	0.17 ^{ns}
42	17.74	17.40	0.50	0.65 ^{ns}	33.04	32.29	0.76	0.52 ^{ns}
43	18.58	18.18	0.61	0.67 ^{ns}	40.86	38.93	1.26	0.34 ^{ns}
44	17.60	17.10	0.55	0.55 ^{ns}	32.54	30.99	0.87	0.28 ^{ns}

^{ns} non-significant

4.7.4 Egg shell quality parameters

The recorded mean values of specific gravity and shell thickness of Gramasree eggs from 33 to 44 weeks of age for two dietary groups are presented in the Table-23.

The calculated mean values of weekly egg surface area (cm²) for two dietary groups are presented in the Table-24.

The recorded and calculated mean values of shell weight, shell per cent, unit surface and mean breaking strength from 33 to 44 weeks in deep litter system are presented in the Table-25 and Table-26.

Table 23. Mean Specific Gravity and Shell Thickness of Gramasree hens from 33 to 44 weeks of age

Age (week)	Specific gravity				Shell Thickness (mm)			
	Groups		SEM	p-value	Groups		SEM	p-value
	G4	G5			G4	G5		
33	1.090	1.093	0.002	0.37 ^{ns}	0.366	0.347	0.02	0.47 ^{ns}
34	1.090	1.097	0.005	0.37 ^{ns}	0.337	0.359	0.02	0.51 ^{ns}
35	1.093	1.093	0.003	1.00 ^{ns}	0.384	0.344	0.02	0.26 ^{ns}
36	1.093	1.093	0.003	1.00 ^{ns}	0.387	0.359	0.01	0.25 ^{ns}
37	1.097	1.090	0.002	0.12 ^{ns}	0.390	0.359	0.01	0.14 ^{ns}
38	1.093	1.100	0.002	0.12 ^{ns}	0.360	0.380	0.01	0.39 ^{ns}
39	1.090	1.097	0.002	0.12 ^{ns}	0.336	0.370	0.01	0.10 ^{ns}
40	1.097	1.093	0.003	0.52 ^{ns}	0.397	0.341	0.01	0.05 ^{ns}
41	1.090	1.093	0.002	0.37 ^{ns}	0.373	0.385	0.02	0.72 ^{ns}
42	1.097	1.093	0.003	0.52 ^{ns}	0.378	0.378	0.01	1.00 ^{ns}
43	1.093	1.093	0.003	1.00 ^{ns}	0.390	0.382	0.00	0.23 ^{ns}
44	1.093	1.090	0.002	0.37 ^{ns}	0.408	0.366	0.02	0.21 ^{ns}

^{ns} non-significant

Table 24. Mean Egg Surface Area of Gramasree hens from 33 to 44 weeks of age (cm²)

Age (week)	Groups		SEM	p-value
	G4	G5		
33	69.01	67.30	2.50	0.65 ^{ns}
34	68.39	67.16	1.31	0.54 ^{ns}
35	70.26	68.83	1.15	0.43 ^{ns}
36	68.37	68.27	1.96	0.97 ^{ns}
37	66.03	72.16	1.66	0.06 ^{ns}
38	67.83	70.90	2.13	0.36 ^{ns}
39	66.71	66.53	0.86	0.89 ^{ns}
40	67.15	65.75	1.89	0.63 ^{ns}
41	66.41	68.13	0.88	0.24 ^{ns}
42	69.11	67.47	1.79	0.55 ^{ns}
43	66.95	69.48	2.17	0.46 ^{ns}
44	66.06	66.86	2.01	0.79 ^{ns}

^{ns} non-significant

Table 25. Mean Shell Weight, Shell Per cent and Shell Weight per Unit Surface Area of Gramasree hens from 33 to 44 weeks

Age (week)	Shell weight (g)				Shell percent (%)				Shell weight per unit surface area (mg/cm ²)			
	Groups		SEM	p-value	Groups		SEM	p-value	Groups		SEM	p-value
	G4	G5			G4	G5			G4	G5		
33	4.94	5.25	0.26	0.44 ^{ns}	9.50	9.13	0.36	0.52 ^{ns}	0.38	0.34	0.04	0.54 ^{ns}
34	5.73	5.04	0.34	0.22 ^{ns}	8.80	9.60	0.55	0.36 ^{ns}	0.34	0.35	0.02	0.61 ^{ns}
35	5.43	5.04	0.38	0.51 ^{ns}	9.80	8.90	0.65	0.39 ^{ns}	0.40	0.35	0.02	0.18 ^{ns}
36	5.73	5.24	0.37	0.40 ^{ns}	10.13	9.30	0.48	0.29 ^{ns}	0.40	0.36	0.03	0.49 ^{ns}
37	5.50	5.59	0.28	0.84 ^{ns}	10.27 ^b	9.20 ^a	0.21	0.02*	0.36	0.41	0.03	0.38 ^{ns}
38	5.35	6.21	0.42	0.22 ^{ns}	9.57	10.47	0.45	0.23 ^{ns}	0.36	0.44	0.04	0.24 ^{ns}
39	5.46	5.41	0.36	0.93 ^{ns}	10.10	10.00	0.79	0.93 ^{ns}	0.36	0.36	0.02	0.92 ^{ns}
40	5.72	5.26	0.27	0.29 ^{ns}	10.43	9.83	0.16	0.06 ^{ns}	0.39	0.35	0.03	0.37 ^{ns}
41	5.33	5.61	0.27	0.50 ^{ns}	9.87	10.00	0.30	0.77 ^{ns}	0.44	0.44	0.01	0.25 ^{ns}
42	5.79	3.90	1.01	0.26 ^{ns}	10.17	7.13	1.93	0.33 ^{ns}	0.40	0.26	0.07	0.22 ^{ns}
43	5.40	5.74	0.46	0.63 ^{ns}	9.80	10.00	0.65	0.84 ^{ns}	0.36	0.40	0.04	0.54 ^{ns}
44	5.68	4.90	0.30	0.14 ^{ns}	10.57 ^b	8.97 ^a	0.38	0.04*	0.37	0.33	0.03	0.31 ^{ns}

^{a, b} Mean values with different superscripts within a row differ significantly

* Significant at p<0.05 ^{ns} non-significant

Table 26. Mean Breaking Strength of eggs of Gramasree hens from 33 to 44 weeks of age (g)

Age (week)	Groups		SEM	p-value
	G4	G5		
33	2059.3	1992.3	97.2	0.65 ^{ns}
34	2034.0	1986.7	50.4	0.54 ^{ns}
35	2106.0	2050.7	44.4	0.43 ^{ns}
36	2034.0	2029.0	75.9	0.97 ^{ns}
37	1943.0	2181.0	65.3	0.06 ^{ns}
38	2012.7	2131.7	82.1	0.36 ^{ns}
39	1969.3	1962.0	32.7	0.88 ^{ns}
40	1986.00	1933.3	71.7	0.63 ^{ns}
41	1957.70	2023.7	33.8	0.24 ^{ns}
42	2062.0	1999.0	69.0	0.55 ^{ns}
43	1979.3	2076.3	83.8	0.46 ^{ns}
44	1945.3	1975.7	76.7	0.79 ^{ns}

^{ns} non-significant

4.8 FERTILITY AND HATCHABILITY OF GRAMASREE BIRDS DURING EXPERIMENTAL PERIOD (per cent)

The calculated mean fertility from 33 to 44 weeks was 95.24 per cent in G4 and 94.83 per cent in G5. The calculated mean hatchability from 33 to 44 weeks was 84.07 per cent in G4 and 82.33 per cent in G5. The weekly mean fertility and hatchability of Gramasree hatching eggs from 33 to 44 weeks of age in deep litter system are presented in the Table 27.

Table 27. Fertility and hatchability of Gramasree birds during experimental period (Per cent)

Age (week)	Fertility (%)				Hatchability (%)			
	Groups		SEM	p-value	Groups		SEM	p-value
	G4	G5			G4	G5		
33	96.85 ^b	94.82 ^a	0.49	0.04*	87.37	85.31	2.05	0.52 ^{ns}
34	97.90	96.44	1.04	0.37 ^{ns}	88.77	84.98	1.96	0.24 ^{ns}
35	95.68	93.89	0.92	0.24 ^{ns}	83.77	83.52	1.22	0.89 ^{ns}
36	96.36	96.48	0.72	0.91 ^{ns}	89.26	84.45	2.75	0.28 ^{ns}
37	92.53	93.89	1.44	0.54 ^{ns}	82.84	83.15	1.74	0.90 ^{ns}
38	93.08	94.01	1.3	0.64 ^{ns}	83.02	80.12	1.17	0.15 ^{ns}
39	94.98	94.63	0.62	0.71 ^{ns}	80.30	80.93	1.53	0.79 ^{ns}
40	96.11	94.51	0.82	0.24 ^{ns}	80.55	79.13	1.48	0.53 ^{ns}
41	94.62	92.45	1.5	0.36 ^{ns}	82.10	80.88	0.74	0.31 ^{ns}
42	94.22	94.10	0.64	0.90 ^{ns}	82.79	83.23	1.78	0.87 ^{ns}
43	93.25	94.97	1.09	0.33 ^{ns}	83.20	82.51	1.36	0.74 ^{ns}
44	92.88	94.75	1.13	0.31 ^{ns}	82.01	81.13	0.88	0.52 ^{ns}

^{a, b} Mean values with different superscripts within a row differ significantly

* Significant at $p < 0.05$ ^{ns} non-significant

4.9 EFFECT OF CALCIUM SUPPLEMENTATION ON TIBIAL CALCIUM

The recorded tibial characteristics and tibial calcium content of Gramasree hens in deep litter and rooster in cages at 44 weeks of age for the five dietary treatment groups are presented in the Table 28.

Table 28. Tibial Characteristics of Gramasree hens and roosters

Treatment	Tibial weight	Tibial length (mm)	Tibial thickness (mm)	Tibial width	Tibial Ash (%)	Tibial calcium (%)
	(g)			(mm)		
Tibial Characteristics of Gramasree roosters						
G1	6.11 ^b	126.27 ^a	6.10 ^a	13.33	53.42	19.66 ^b
G2	6.80 ^a	126.40 ^a	6.53 ^a	13.33	56.52	20.96 ^b
G3	5.58 ^c	120.90 ^b	5.97 ^b	13.07	55.82	26.58 ^a
SEM	0.001	0.27	0.07	0.39	2.16	0.61
p-value	0.00**	0.00**	0.00**	0.86 ^{ns}	0.59 ^{ns}	0.00**
Tibial Characteristics of Gramasree hens						
G4	5.59	113.13	5.86	12.37	51.69	20.34
G5	6.29	110.33	5.90	12.50	55.54	22.75
SEM	0.82	4.34	0.20	0.38	1.78	3.12
p-value	0.58 ^{ns}	0.67 ^{ns}	0.91 ^{ns}	0.82 ^{ns}	0.20 ^{ns}	0.62 ^{ns}

^{a, b} Mean values with different superscripts within a column differ significantly

** Significant at $p < 0.01$ ^{ns} non-significant

4.10 EFFECT OF CALCIUM SUPPLEMENTATION ON SERUM CALCIUM CONTENT

The recorded mean serum calcium and phosphorus values of Gramasree roosters housed in cage (mg/dL) for three dietary treatment groups are presented in the Table-11. The recorded mean values of serum calcium and phosphorus of Gramasree hens housed in deep litter (mg/dL) for two dietary treatment groups are presented in the Table-29.

Table 29. Mean values of serum calcium and phosphorus of Gramasree hens housed in deep litter (mg/dL)

Age (week)	Calcium(mg/dL)				Phosphorus(mg/dL)			
	Groups		SEM	p-value	Groups		SEM	p-value
	G4	G5			G4	G5		
33	15.33	13.33	0.53	0.06 ^{ns}	10.41	8.59	2.03	0.56 ^{ns}
34	14.00	13.33	2.05	0.83 ^{ns}	10.90	7.61	1.20	0.13 ^{ns}
35	21.33 ^b	18.33 ^a	0.53	0.02*	15.44	7.07	2.24	0.06 ^{ns}
36	17.33	15.67	2.19	0.62 ^{ns}	5.76	8.82	1.54	0.23 ^{ns}
37	17.00	12.33	2.21	0.21 ^{ns}	10.61	9.18	1.43	0.52 ^{ns}
38	12.67	15.00	1.89	0.43 ^{ns}	7.62	7.75	1.03	0.93 ^{ns}
39	12.67	14.00	1.65	0.60 ^{ns}	6.37	9.58	1.52	0.21 ^{ns}
40	14.33	15.00	1.11	0.69 ^{ns}	7.81	6.86	1.35	0.65 ^{ns}
41	16.33	13.67	1.99	0.40 ^{ns}	11.29	9.31	2.87	0.65 ^{ns}
42	19.67 ^b	13.67 ^a	1.39	0.04*	11.08	8.28	1.76	0.32 ^{ns}
43	17.33	19.67	1.76	0.40 ^{ns}	6.41	10.42	1.44	0.12 ^{ns}
44	19.00	15.67	1.97	0.30 ^{ns}	9.69	8.66	2.49	0.78 ^{ns}

^{a, b} Mean values with different superscripts within a row differ significantly

** Significant at $p < 0.01$

* Significant at $p < 0.05$

^{ns} non-significant

4.11 EFFECT OF CALCIUM SUPPLEMENTATION ON TECHNO-ECONOMICS

The data on techno-economics of Gramasree breeder study on production of hatching eggs during experimental period from 32 to 44 weeks of age for five dietary treatment groups are presented in the Table 30.

Table 30. Techno-Economics of Hatching Egg Production

Parameters	Groups	
	G4	G5
Technical details		
Cost of Breeder Chicken feed (Rs/kg)	32.03	32.03
Feed consumed (Kg)	1987.50	1998.50
Expenditure		
Total egg shell and shell grit consumed (kg)	82.30	53.99
Total feed cost (Rs. 32.03 /kg)	63659.63	64011.95
Calcium source cost (Rs. 3 /kg)	246.90	269.94
Overhead cost (Rs. 5 /kg)	925.00	930.00
Total expenditure (Rs)	64831.53	65211.89
Income		
Total no. of hatching eggs	8405.00	8050.00
Cost per egg (Rs)	7.74	8.15
Income per egg (Rs)	15.00	15.00
Profit per egg (Rs)	7.26	6.85

Table 31. Mean feed intake and calcium intake of Gramasree birds in deep litter during the experimental period

Parameter	Groups		SEM	P-value
	G4	G5		
Feed intake (g/day/bird)	124.33	121.42	4.03	0.67 ^{ns}
Egg shell powder(G4) and shell grit(G5) (g/day/bird)	5.06 ^a	3.20 ^b	0.16	0.02*
Calcium intake from feed (g/day/bird)	4.56	4.45	0.15	0.66 ^{ns}
Calcium intake from egg shell powder and shell grit (g/day/bird)	2.04	1.39	0.06	0.03 ^{ns}
Total calcium intake (g/day/bird)	6.60	5.84	0.19	0.08 ^{ns}

^{a, b} Mean values with different superscripts within a row differ significantly

* Significant at $p < 0.05$ ^{ns} non-significant

Table 32. Mean egg production, egg quality, serum parameters and body weight of Gramasree birds in deep litter during the experimental period

Parameter	Groups		SEM	p-value
	G4	G5		
Hen-Day Egg Production (%)	57.4	54.33	3.37	0.81 ^{ns}
Hen-Housed Egg Production (%)	53.04	53.54	0.97	0.53 ^{ns}
Feed conversion ratio (per egg mass)	2.84	2.98	0.18	0.60 ^{ns}
Feed conversion ratio (per dozen eggs)	4.14	4.23	0.27	0.54 ^{ns}
Fertility (%)	94.87	94.58	0.98	0.50 ^{ns}
Hatchability (%)	83.83	82.45	1.56	0.57 ^{ns}
External egg quality				
Egg weight (g)	53.09	53.59	0.95	0.50 ^{ns}
Egg length (mm)	54.24	54.44	1.02	0.65 ^{ns}
Egg width (mm)	42.95	163.33	85.99	0.55 ^{ns}
Shape Index	78.75	77.3	1.29	0.36 ^{ns}
Internal egg quality				
Albumen Height (mm)	6.577	6.772	0.17	0.48 ^{ns}
Haugh unit	83.06	83.26	1.72	0.57 ^{ns}
Yolk Colour (grade)	7.61	7.42	0.49	0.35 ^{ns}
Yolk height (mm)	16.76	17.02	0.5	0.44 ^{ns}
Yolk index	39.14	40.4	1.43	0.44 ^{ns}
Yolk Weight (g)	17.46	17.17	0.74	0.54 ^{ns}
Yolk percent (%)	33.33	32.81	1.47	0.50 ^{ns}
Egg shell quality				
Specific gravity	1.093	1.094	0.003	0.49 ^{ns}
Shell Thickness (mm)	0.38	0.36	0.01	0.36 ^{ns}
Shell weight (g)	5.51	5.27	0.39	0.45 ^{ns}
Shell percent (%)	9.92	9.38	0.58	0.40 ^{ns}
Shell weight per unit surface area (mg/cm ²)	0.38	0.37	0.03	0.44 ^{ns}
Serum parameters				
Calcium (mg/dl)	15.6	14.77	1.61	0.38 ^{ns}
Phosphorous (mg/dl)	9.45	8.51	1.74	0.43 ^{ns}
Gramasree hens body weight in deep litter				
32 weeks (kg)	1.94	1.96	0.03	0.66 ^{ns}
44 weeks (kg)	2.09	2.06	0.03	0.49 ^{ns}
Gramasree roosters body weight in deep litter				
32 weeks	2.56	2.63	0.15	0.76 ^{ns}
44 weeks	2.61	2.54	0.07	0.57 ^{ns}

^{ns} non-significant

5. DISCUSSION

The results obtained in the present study of supplementing different calcium sources to Gramasree breeder birds are discussed in this chapter under the following headings

5.1 CHEMICAL COMPOSITION, PARTICLE SIZE AND *IN-VITRO* SOLUBILITY OF CALCIUM SOURCES

The chemical composition of egg shell is presented in Table-4. Egg shell without shell membrane was sundried and analysed for chemical composition and reported as 8.30 per cent crude protein, 42.16 per cent calcium and 1.70 per cent phosphorus. Like that, Islam (2021) reported kitchen-extruded egg shell contains 4.24 per cent protein, 29.75 per cent calcium and 14.82 per cent available phosphorus and the hatchery-extruded egg shell contains 13.80 per cent crude protein, 25.53 per cent calcium and 13.87 per cent available phosphorus. They clarified that in extruded processing egg shell was boiled at 100° C and sundried before using, which is different to our processing method.

The estimated crude protein in the present study was higher than kitchen-extruded egg shell and lesser than hatchery-extruded egg shell reported by Islam (2021), however lesser value of 2.14 per cent crude protein was reported by Lertchunhakiat *et al.* (2016). Olgun *et al.* (2015) reported 32.3 per cent of calcium content in egg shell, but in our study calcium content was estimated as 42.16 per cent on dry matter basis which was higher than the previously mentioned values by 33.5-34.8 per cent Ca in eggshell cited from Muir *et al.* (1976). The difference in nutritive value of egg shell might be due to source of collection and processing involved, present study materials were collected from bakery which are all infertile eggs and as explained by Islam (2021), egg shells from hatchery was lower in calcium and phosphorus content because of calcium and phosphorus utilization by embryo during incubation.

The dietary calcium requirement for laying hens were highly influenced by particle size, solubility and source of calcium added in the feed and supplemented as extra calcium source. The relative proportion of coarse particle (>2.00 mm) in egg shell powder (84.13 per cent) was comparatively more than shell grit (69.11 per cent). The medium size particle (0.75 to 2.00 mm) in shell grit was 22.56 per cent. Meanwhile, the fine particles (<0.75 mm) are very less in egg shell supplemented to the laying hens and roosters.

The *in-vitro* solubility of coarse egg shell and shell grit used in this study was 40.61 ± 0.84 and 56.21 ± 1.04 per cent, respectively. Pizzolante *et al.* (2011) studied the effect of different calcium sources with different particle size and solubility and used particle size of 0.44, 2.40 and 2.19 mm for fine lime stone, coarse limestone and oyster shell, respectively and mentioned *in-vitro* solubility of 31 per cent for fine limestone powder, 28 per cent was for coarse limestone and 46 per cent for oyster shell.

In this study, coarse egg shell powder has lesser *in-vitro* solubility of 40.61 per cent compared to fine powder with 55.39 ± 0.84 per cent. similar to that of Pelicia *et al.* (2011) findings reported in limestone powder. The influence of particle size and solubility of calcium sources like egg shell powder, oyster shell, lime stone powder on egg production performance and egg quality were studied by different researchers and found that egg shell powder with particle sizes of 3-5 mm showed better performance. Further, explained that coarse and less soluble calcium sources were recommended to maintain adequate blood calcium level, because readily solubilised fine particles were not available in the digestive tract of poultry at night hours for eggshell synthesis and (Miles, 2000).

The chemical composition of egg shell and shell grit are presented in the Table-3. The calcium and phosphorus content of egg shell powder are 42.16 ± 0.48 and 1.70 ± 0.32 per cent, respectively. Further, results obtained shell grit used in this study in scanning electron microscope revealed that 3.41 per cent mass of aluminum and 96.06 per cent of calcium, which is not identified in egg shell

powder. However, Olgun *et al.* (2015) egg shell powder as 32.3 per cent calcium and 7.6 per cent phosphorus and oyster shell contained 32.09 per cent calcium and 1.09 per cent phosphorus.

5.2 EFFECT OF CALCIUM SUPPLEMENTATION IN MALE BIRDS

5.2.1 Effect of calcium supplementation on feed intake, calcium intake and body weight in Gramasree roosters housed in cage

The overall mean feed intake of the roosters in present study are 87.58, 86.86 and 88.28 g/rooster/day, in control, egg shell and shell grit groups, respectively and shown in the Table-7. As age increased, feed intake in G1 groups are increased and in other groups that increase are not recorded. Like that, the overall mean total calcium intake recorded in the study are 3.46, 3.49 and 3.25 g/rooster/day in control, egg shell and shell grit group, respectively and shown in the Table-8. The free choice feeding of egg shell powder and shell grit had influenced the body weight, feed intake and calcium intake and there was a decrease in feed intake ($p < 0.01$) and calcium intake compared to control group roosters. The body weight was reduced in shell grit group compared to other two groups.

In contrary to the present findings, Gongruttananun (2011a) reported that the feed intake was more in egg shell group (118.56g/rooster/day) and Lertchunhakiat *et al.* (2016) also reported that the egg shell powder inclusion at 10.8 per cent level did not influence the feed intake and body weight compared to other group of Arbor Acers breeder cocks.

5.2.2 Effect of calcium supplementation on semen quality parameters

The semen quality parameters studied during the experimental period are presented in Table-9 and Table-10. The effects of free choice feeding of egg shell powder and shell grit on semen quality parameters are compared.

The average semen volume of G1, G2 and G3 group roosters are 0.13, 0.16 and 0.14 ml/rooster, respectively and Chauhan *et al.* (2009) reported in Gramapriya cocks that the average ejaculate volume was 0.39 ± 0.02 ml/rooster with sperm concentration and total sperm count of $2.49 \pm 0.03 \times 10^9$ / ml and $0.98 \pm 0.04 \times 10^9$ /ejaculate. Whereas, weekly mean semen concentration recorded in this study was 4.25 , 5.49 and 5.31×10^9 / ml in control, egg shell and shell grit groups, respectively and the values are higher than Chauhan *et al.* (2009) reported values. However, no significant differences were found in semen concentration between the groups and agreed to the results of Shanmugam *et al.* (2012) and Lertchunhakiat *et al.* (2016).

Similarly, Gongruttananun (2011a) and Lertchunhakiat *et al.* (2016) reported the semen volume as 0.37 ml/ejaculate and 0.39 ml/ejaculate, respectively and they also reported that source of calcium not affected the semen volume, which is in accordance with present findings. Meanwhile, semen colour average score evaluated during this study are 2.35, 2.80 and 2.83 and results are similar to findings of Shanmugam *et al.* (2012) and Lertchunhakiat *et al.* (2016).

The average semen motility reported in present study are 47.92, 61.04 and 61.46 per cent in control, egg shell and shell grit groups, respectively and no significant difference in semen motility between the groups are reported except 36, 39 and 40th weeks. Similar findings were reported by Shanmugam *et al.* (2012) and Lertchunhakiat *et al.* (2016).

5.3 EFFECT OF CALCIUM SUPPLEMENTATION IN GRAMASREE BIRDS

5.3.1 Effect of calcium supplementation on feed intake, calcium intake and body weight of Gramasree birds

The present experimental values on feed intake, calcium intake and body weight are summarized in Table-13, Table-14 and Table-32, respectively. It was found that, free choice feeding of calcium sources like egg shell powder and shell grit did not show any influence on feed intake and similar between the groups.

Like that, similar results to the present findings are reported by Lee *et al.* (2021) who replaced limestone powder with coarse egg shell in layer diet did not influence feed intake. The other researcher Olgun *et al.* (2015) replaced limestone powder and egg shell powder in layer ration fed to 25-week-old and found no difference between the limestone and egg shell and shell grit fed group in their feed intake and feed efficiency.

In contrary, Kismiati *et al.* (2012) reported there was significant increase in feed intake, while egg shell waste was supplemented at 7.5 per cent of the diet, compared to other brown laying hens' group. However, the egg shell powder intake recorded in the present study was increased from 3.36 per cent at 32nd week to 7.10 per cent at 44th week, but shell grit intake was 2.55 per cent at 32nd week to 4.77 per cent at 44rd week of age and significant difference was found between the groups. Similar to our findings, Lee *et al.* (2021) elucidated supplementation of egg shell instead of limestone powder did not affect the feed intake and similar feed intake was found between groups.

Birds are able to adjust their nutrient intake through feed intake, however the feed intake depends on the energy content of the feed and ICAR 2013 recommendations of calcium for breeder hens are 3.0 per cent and it is expected that the calcium requirements could be met by difference in their feed intake. However, in the present study mean calcium intake through feed from 32 to 44 weeks was 4.56 g/bird/day in G4 group and found that average 2.04 g of calcium was consumed through egg shell fed out of total calcium (6.60 g) consumed by the Gramasree birds and indicated that 30.34 per cent of the more calcium dietary supplementation is required. Moreover, calcium intake through feed was not differing significantly between the groups and difference in calcium intake through calcium sources. There was significantly ($p < 0.01$) lesser quantity of shell grit was consumed and calcium intake was also lesser during the experimental period.

Rathnayaka *et al.* (2020) conducted a calcium supplementation by free choice feeding claimed that more calcium intake was recorded in Oyster shell (5.96 g/bird/day), limestone powder (6.27 g/bird/day) group compared to bone meal supplemented group. However, Ahmed *et al.* (2013) reported that there was significant increase in feed intake in limestone group (142.02 g/hen/day) compared to oyster shell group (139.53 g/hen/day). Pastore *et al.* (2012) reported that the feed intake was not affected by different levels of calcium and difference in calcium and phosphorus ratio of the diet.

Meanwhile, no significant difference in body weight between the groups was recorded in this study in hens and roosters maintained in deep litter system and similar observations also reported by Olgun *et al.* (2015) that the supplementation of egg shell powder and oyster shell had no significant change in body weight of the laying hens.

5.4 EFFECT OF CALCIUM SUPPLEMENTATION ON EGG PRODUCTION

5.4.1 Hen-Day and Hen-Housed egg production of Gramasree layers during experimental period

The mean hen day (HD) and hen housed (HH) egg production in egg shell powder supplemented group was 57.40 and 53.04 per cent, respectively. Like that, 54.33 and 53.54 per cent, HD and HH production, respectively was recorded in this during 33nd-44th weeks of age and shown in the Table-15. The other researcher, Valavan *et al.* (2016) studied the Gramapriya bird's production performance under intensive system and reported that age at first egg was 175.52 days, hen day egg production was 50.60 per cent and weight at 40th weeks of age was 58.10 g/bird. Like that, hen day and hen housed egg production in per cent were recorded as 35.53 and 32.46 per cent for Gramalashimi laying hens and 34.3 and 32.44 per cent in Gramasree laying hens (Ajithbabu, 2010).

While, comparing egg shell powder supplementation as calcium source, Gongruttananun (2011c) supplemented egg shell powder as calcium source in 144

Rhode Island Red hens at 25 weeks of age, used egg shell powder as calcium source and recorded 68.28 per cent of egg production, which was similar to limestone powder source group birds.

Meanwhile, Kismiati *et al.* (2012) also commented that egg production and egg weight in 7.5 per cent egg shell waste fed group shown significantly increased egg production (91.42 per cent) and egg weight (55.73 g/egg) and increase in feed, protein, calcium and phosphorus intake also. But, in our study, egg shell powder received birds had not increased the egg production and showed similar results of other group birds.

5.4.2 Mean egg mass and feed conversion ratio of Gramasree layers

The present experimental findings revealed that free-choice feeding of egg shell powder and shell grit had not influenced the egg weight and egg mass of hatching eggs. The egg weight was recorded as 53.09 g for egg shell group and 53.59 g for shell grit groups. The egg mass was 30.06 g hen/day for egg shell powder and 28.87 g/hen/day for shell grit supplemented group. However, Lee *et al.* (2021) stated that use of coarse egg shell powder with 3-5 mm particle size had significantly ($p < 0.01$) increased the egg weight and reported egg mass ($p < 0.05$) value of 37.64 g/hen/day and decreased feed conversion ratio ($p < 0.01$) as compared to other groups received limestone powder and shell grit.

The average values of feed conversion ratio per kg egg mass in G4 and G5 are 2.84 and 2.99, respectively. Like that, the average feed conversion ratio per dozen eggs recorded are 4.14 and 4.23, in G4 and G5, respectively. However, feed conversion ratio per kg and dozen eggs were not differing significantly (Table-17).

Pelicia *et al.* (2011) reported that the feed conversion ratio for dozen eggs calculated were 1.74 (T1), 1.769 (T2) and 1.77 (T3). Pizzolante *et al.* (2011) in his study reported that the feed conversion ratio for dozen eggs and kg eggs as 1.79 and 2.10, respectively. Houndonougbo (2012) also reported that the feed conversion ratio per kg egg mass was poorer in snail shell supplemented group

compared to oyster shell group. Ahmed *et al.* (2013) also reported that feed efficiency reported in oyster shell group (2.99) was significantly better than limestone powder (4.66) group during the experimental period. The higher values in the present study were due to breed differences.

5.5 EFFECT OF CALCIUM SUPPLEMENTATION ON EGG QUALITY CHARACTERISTICS

5.5.1 External egg quality parameters

The free choice feeding of egg shell powder and shell grit as calcium sources on external egg quality parameters like egg weight (g), egg length (mm), egg width (mm) and shape index are presented in Table-18 and Table-19. The overall mean values of shape index calculated were similar to findings of Sinha *et al.* (2017) who measured 77.72 per cent in Gramapriya laying hens and egg length (51.66 mm) and width (40.10 mm) similar to the present study values.

The influence of egg shell on external egg qualities were not reported in present study and similar to shell grit group. Ahmed *et al.* (2013) mentioned that feeding of oyster shell in place of limestone powder not affected the egg length and width. Like that, Olgun *et al.* (2015) recorded that while limestone was replaced by egg shell powder had similar values of external egg quality parameters in laying hens.

5.5.2 Internal egg quality characteristics

5.5.2.1 Albumen Height

The recorded mean values of albumen height are 6.64 and 6.70 mm in G4 and G5, respectively. There was no significant difference ($p > 0.05$) in albumen height between the groups except 35 and 43 weeks as shown in Table-20. Sinha *et al.* (2017) reported the albumen height as 5.87 mm in Gramapriya eggs. According to Lee *et al.* (2021), feeding of oyster shell and egg shell (fine and coarse) had significantly ($p > 0.05$) increased the albumen height (7.65 and 7.62

mm, respectively), compared to cockle shell. However, Ahmed *et al.* (2013) reported that calcium level had no influence on albumin height. Like that, Rathnayaka *et al.* (2020) studied that calcium supplementation had no effect ($p>0.05$) on albumen quality parameters of eggs and none of the internal egg quality parameters were affected ($p>0.05$) by the different calcium sources. Gongruttananun (2011c) conducted a study in laying hens with feeding 9.06 per cent egg shell reported 6.62 mm albumen height, similar to present study values.

5.5.2.3 Haugh Unit Score

The mean Haugh unit values from 33-44th weeks are 83.17 and 82.86 for egg shell powder and shell grit group, respectively. In agreement to present results, Sinha *et al.* (2017) reported Haugh Unit as 81.35 for Gramapriya eggs. There was no significant difference ($p>0.05$) in Haugh unit recorded between the groups except at 43rd week. Rathnayaka *et al.* (2020) reported that similar Haugh Unit score was measured for different sources like limestone powder, oyster shell and bone meal as calcium supplementation had not affected Haugh Unit ($p>0.05$) and similar results were recorded in present study.

5.5.2.4 Yolk Colour

In present study, the mean yolk colour score recorded for egg shell powder group was 7.33 and in shell grit group was 6.33 at 40 weeks, however no significant difference was found between the groups. Padhi *et al.* (2014) reported that more intense yolk color was measured at 64th week of age, and significantly lesser intense colour was recorded at 28th week in Vanaraja laying birds. The average yolk colour measured at 40th of week in Vanaraja birds was 7.45. Lee *et al.* (2021) reported that different calcium sources not significantly affected the yolk colour in brown egg laying hens. Similarly, Gongruttananun (2011c) reported 5.94 yolk colour score in egg shell powder supplemented laying hens in place of limestone powder and not significantly differed to other group of birds received limestone powder and mixture of egg shell and limestone powder.

5.5.2.5 Yolk Height

The weekly mean yolk height is presented in the Table-21. The average yolk height (mm) recorded at experimental period from 33 to 44 weeks was 16.76 and 17.02 in egg shell powder and shell grit groups, respectively. SenthilMurugan (2017) reported the yolk height as 19.48 mm in Gramasree laying hens. Like that, Sinha *et al.* (2017) measured 16.44 mm of yolk height in Gramapriya birds. The results of the present study on yolk height revealed, different calcium sources are not significantly influenced the yolk height.

5.5.2.6 Yolk Index

The yolk index values calculated in egg shell and shell grit was not significantly different between the groups and the overall mean values of yolk index are 39.14 and 40.40 per cent in egg shell and shell grit group eggs, respectively. The reported values in Vanaraja, an Indian improved variety laying birds by Sinha *et al.* (2017) was 43.88 per cent and 45.11 per cent was reported by SenthilMurugan (2017). Padhi *et al.* (2014) reported the yolk index was significantly different at different weeks of age. However, the difference was not evidenced from 40th to 72nd weeks of age in Vanaraja birds. In the present study, there was no significant difference in yolk index between the groups except at 33 and 38th weeks of age. Rathnayaka *et al.* (2020) reported that calcium supplementation had no effect ($p>0.05$) on yolk quality parameters of eggs and none of the internal egg quality parameters were affected ($p>0.05$) by the different calcium sources.

5.5.2.7 Yolk Weight

Sinha *et al.* (2017) reported the yolk weight as 12.692 g in Gramapriya birds and SenthilMurugan (2017) recorded 17.08 g in Gramasree birds. Padhi *et al.* (2014) mentioned that yolk weight increases as age increases along with egg weight significantly in Vanaraja laying line. In accordance to the other findings, calculated values of mean yolk weight recorded in this study was 17.46 g and

17.17 g for egg shell and shell grit, respectively and did not differ significantly. Similarly, Ahmed *et al.* (2013) reported that different calcium level and sources had no influence on yolk weight. Further, Padhi *et al.* (2014) yolk weight had positive correlation with egg weight.

5.5.2.8 Yolk Ratio

The average yolk ratio from 33 to 44 weeks was 33.33 and 32.81 per cent, respectively among treatment groups (Table-21). There was no significant difference ($p > 0.05$) in yolk ratio between the groups. Similar results were obtained by Sinha *et al.* (2017) who reported the yolk ratio as 31.32 per cent.

5.5.3 Egg shell quality parameters

Egg quality traits like egg shell thickness, shell weight, surface area and breaking strength are directly correlated with the egg specific gravity. The values on egg specific gravity and egg surface area indicated egg shell and internal quality.

5.5.3.1 Specific Gravity

The mean specific gravity is presented in the Table-23. The recorded values of mean specific gravity are 1.093 and 1.094 for egg shell powder and shell grit, respectively. During the experimental period, there was no significant values recorded for specific gravity between the groups. The results of the present study are inconsistent with the findings of Pelicia *et al.* (2009); Saunders-Blades *et al.* (2009) and Tunc and Cufadar (2015) who studied the effect of feeding different calcium sources like limestone powder, oyster shell and egg shell with different particle size. However, Bingfan *et al.* (2017) reported specific gravity of egg was significantly increased at higher intake of dietary calcium intake at 5.0 g/day/hen. In our study, the recorded calcium intake through feed and free-choice feeding of calcium sources are 6.60 and 5.84 g/bird/day in egg shell powder and shell grit group birds.

5.5.3.2 Shell Thickness

The average shell thickness recorded in the present study are 0.38 and 0.36 mm for egg shell and shell grit fed groups, respectively. The results are correlated with Gongruttananun (2011c) and Ahmed *et al.* (2013) who reported that different sources of calcium not affecting the shell thickness. The egg laying varieties like Gramapriya birds had recorded 0.37 mm as shell thickness (Sinha *et al.*, 2017).

5.5.3.3 Shell Weight (g) and Shell Per cent

The recorded mean shell weight in this study are 5.51 g and 5.27 g for egg shell powder and shell grit group, respectively and not differing significantly. These findings were similar to Gongruttananun (2011c) and Ahmed *et al.* (2013) findings in laying hens. Moreover, Sinha *et al.* (2017) reported 4.458 g in Gramapriya birds. On studying the factors influencing the shell weight, it was understood that shell weight increases as age increases and differ significantly ($p < 0.05$) (Padhi *et al.*, 2014).

Like that, shell per cent calculated in this study is presented in Table-25. The overall shell per cent are 9.92 and 9.38 per cent for egg shell and shell grit groups, respectively and found similar values between groups. These findings were correlated with results reported by Pelicia *et al.* (2009). However, Sinha *et al.* (2017) reported that the shell per cent of Gramapriya as 17.925 per cent. Padhi *et al.* (2014) revealed that shell per cent was negatively correlated with egg weight which indicated, higher egg weight has lower shell per cent.

5.5.3.4 Egg Surface Area and Shell Weight per Unit Surface Area

The weekly mean egg surface area is presented in Table-25. The mean calculated egg surface area is 67.69 for egg shell powder and 68.24 cm² for shell grit, respectively and not differing significantly. Like that the calculated average egg shell weight per unit surface area (ESWSA) are 0.38 and 0.37 mg/cm² for egg shell powder and shell grit group, respectively. In contrary, Pelicia *et al.* (2009)

reported dietary calcium levels influenced ESWSA and measured 84.90 mg/cm² at 4.5 per cent calcium supplemented diet and Bingfan *et al.* (2017) mentioned ESWSA was significantly increased at higher intake of dietary calcium at 5.0 g/day/hen. However, in the present study highest calcium intake is recorded at 7.10 g/day/bird in egg shell powder group at 41st weeks of age and significantly ($p < 0.01$) more than shell grit (4.70 g/day/bird), but had similar egg surface area. The consumed calcium in egg shell powder might not be digested properly, not available to the birds.

5.5.3.5 Breaking Strength

The mean egg breaking strength from 33 to 44 weeks was 2007.39 and 2028.44 g. There was no significant difference ($p > 0.05$) in egg breaking strength between the groups and shown in the Table-26. Olgun *et al.* (2015) reported that egg breaking strength (kg) reported was ranged between 4.02 to 4.24 kg respectively.

5.6 FERTILITY AND HATCHABILITY OF GRAMASREE BIRDS

The egg quality factors like egg weight, shell thickness and shape index influences fertility and hatchability (King'ori, 2011). In the present study, the external and internal qualities were recorded similar in both egg shell powder and shell grit supplemented groups and were not differ significantly between the groups. As expected, there was no significant differences were recorded in fertility and hatchability between the groups except at 33rd week. The recorded overall fertility and hatchability per cent was 95.24 and 94.83, respectively for egg shell powder and shell grit groups. Like that, mean hatchability per cent for experimental period was 84.07 and 82.33 per cent in egg shell powder and shell grit, respectively.

Gongruttananun (2011b) reported that there were no significant effects on fertility and hatchability with respect to calcium source. Patra *et al.* (2016) explained that, egg weight had influenced fertility and hatchability per cent in

Vanaraja and Gramapriya laying hens. Further, clarified that medium sized eggs (52.0 -65.0 g) compared to large sized (≥ 65.0 g) and small sized eggs (≤ 52.0 g).

5.7 EFFECT OF CALCIUM SUPPLEMENTATION ON SERUM CALCIUM AND TIBIAL CHARACTERISTICS OF GRAMASREE BIRDS

5.7.1 Effect of calcium supplementation on Tibial characteristics

This experiment investigated effects of free-choice feeding of calcium supplementation in Gramasree hens and roosters on calcium intake, serum calcium and tibial bone characteristics. The layer ration fed to the birds was prepared with 3.0 per cent calcium and 1.0 per cent phosphorus. The intake of calcium and other nutrients were decided by energy content of the feed and intake which were regulated by the hens and roosters. During the experiment period, the calcium intake through feed was 4.56 and 4.45 g/day/bird from egg shell powder and shell grit groups, respectively and not differing significantly. However, the calcium intake through egg shell powder feeding provides 2.04 g/day/bird and shell grit feeding provides 1.39 g/day/bird.

In this study, it was recorded that feed and calcium intake was similar between the groups. The egg production, egg weight and feed efficiency were also similar between the groups. Moreover, during laying cycle, hens required 2.0 to 2.5 g of calcium/egg and two third of the calcium requirement will be met from feed and remaining one third mobilized from medullary bone (Lee *et al.*, 2021). From this study, it could be clarified that egg shell powder and shell grit coarse particle size with more than 2 mm might be stay longer in gizzard because of their slow passage in gastro intestinal tract, which in turn might increases *in-vivo* solubility and calcium availability to the birds.

Further, it was evident that tibial characteristics and serum calcium levels were not differing between the groups. Gongruttananun (2011c) reported that the tibial weight of 8.10 g/kg of body weight and ash content of 54.19 per cent in egg

shell powder supplemented group, in agreement to the present study values of 5.59 g of tibial weight and 51.69 per cent of ash in laying hens.

An *et al.* (2016) reported that the tibial calcium content of 17.79 to 18.25 per cent in their study which was similar to our results, 19.6 to 26.5 per cent. The other researcher, Lee *et al.* (2021) reported that the tibial weight of 12.31 g/100 g body weight, 118.4 mm of length, 7.33 mm tibial width in coarse egg shell supplemented groups. Pelicia *et al.* (2009) further clarified that tibial calcium content was influenced by different dietary calcium levels, however, in the present study total calcium intake was increased as age increases from 4.72 g/day/bird at 33rd weeks to 7.54 g/day/bird at 45th weeks of age in egg shell powder supplemented group and significantly differ at 35th to 40th weeks of age, but tibial ash and calcium content in this study was not differed at 45th weeks of age in laying hens.

The free choice feeding of egg shell powder and shell grit significantly affected the feed intake and calcium sources intake and calcium intake in Gramasree roosters, which did not significantly affect the semen quality characteristics, but tibial calcium content and body weight was significantly different in shell grit supplemented group compared with other two groups.

5.7.2 Effect of calcium supplementation on serum Calcium and Phosphorus content

The serum calcium and phosphorus can be used to evaluate the calcium metabolism in birds, further by correlating with egg production, egg quality, tibia bone characteristics, thereby assessing effect of different calcium sources in laying hens. Serum calcium and phosphorus levels considered as essential important serum minerals in layers during laying cycle. The metabolism of calcium and phosphorus is regulated by dietary levels of calcium and phosphorus and their ratio maintained and the actions of intestine, kidney and skeleton. The values estimated in this study were similar to previously reported values of 16.20 mg/dL of calcium and 8.40 mg/dL of phosphorus by Gongruttananun (2011) in

egg shell powder fed group. The serum calcium and phosphorus content analysed at weekly interval in laying hens and roosters housed in cages and in deep litter were presented in the Table-11 and Table-29, respectively. There was significant difference ($p < 0.05$) in phosphorus content in 33rd, 36st, 41st and 44th weeks between the groups.

An *et al.* (2016) clarified that serum calcium levels were not influenced by different dietary calcium levels, further it was estimated as 29.0 mg/dL of calcium and 6.11 mg/dL of phosphorus at 4.7 per cent dietary calcium levels. However, in contrary Pelicia *et al.* (2009) clarified that different calcium levels in diet influences the serum calcium level and estimated as 228 mg/L, 215 mg/L and 227 mg/L in 3.0, 3.75 and 4.5 per cent calcium supplemented group at 35th weeks of age, respectively.

5.8 EFFECT OF CALCIUM SUPPLEMENTATION ON HATCHABLE EGG PRODUCTION COST AND PROFITABILITY

The production cost for producing hatching eggs by supplementing egg shell and shell grit are presented in the Table-30. According to Houndonougbo (2012) who reported that extra supplementation of oyster shell and snail shell increased the cost of feeding and not economically beneficial compared to limestone powder feeding. However, Islam (2021) pointed out that, the production cost for producing 12 eggs was Rs. 69.61 equals to Rs. 61.74, like that production cost for one kg was 87.36 equals to Rs. 77.48 and claimed that addition of hatchery-extruded egg shell significantly ($p < 0.01$) increased profit and Rs. 32.39 net profit was estimated per dozens of eggs, which equals to Rs. 28.73, compared to other treatments.

6. SUMMARY

The present study was conducted to assess the effect of supplementing egg shell and shell grit as free-choice calcium source on semen quality, egg production, egg quality, fertility and hatchability in Gramasree breeder birds. In experiment I, eighteen Gramasree roosters of 32 weeks were selected based on their body weight (above 2 kg body weight) from Poultry farm, Instructional Livestock Farm Complex (ILFC), College of Veterinary and Animal Sciences (CVAS), Pookode for semen quality studies and they were randomly assigned to three experimental groups *viz.*, G1, G2 and G3 with six birds in each group and separately housed in male cages. In experiment II, 360 breeder hens and 36 roosters of 32 weeks were selected based on their body weight for egg production and egg quality studies and they were randomly allotted to G4 and G5 experimental groups with three replicates with 60 birds of female and six birds of male in each replicate, housed in deep litter system. The experiments I and II were conducted simultaneously at same period between March to May 2021.

The egg shells were collected from local market, processed and the membrane was removed before powdering into a coarse particle size of more than 2 mm. Similarly, shell grit with more than 2 mm particle size was used. The experimental layer breeder ration (control) was prepared as per ICAR, (2013) recommendations and fed to birds in cages and deep litter. The egg shell has 8.30 per cent crude protein, 42.16 per cent calcium and 1.70 per cent phosphorus and shell grit was having 7.47 per cent crude protein, 39.11 per cent calcium and 3.46 per cent phosphorus. The relative proportion of coarse particle (>2.00 mm) in egg shell powder (84.13 per cent) comparatively more than shell grit (69.11 per cent) and given as free choice feeding. The *in-vitro* solubility of coarse egg shell and shell grit used in this study was 40.61 and 56.21 per cent, respectively.

In experiment-I, free choice feeding of egg shell powder and shell grit had influenced the body weight, feed and calcium intake in this study.

There was increased feed intake ($p < 0.01$) in treatment group birds and average calcium intake from egg shell powder and shell grit intake was only 0.26 and 0.30 g/bird/day, respectively and similar among treatments. The average semen volume of G1, G2 and G3 group roosters during the experimental period was 0.13, 0.16 and 0.14 ml/rooster, respectively. The mean semen concentration recorded was 4.25, 5.49 and 5.31 billion per ml in control, egg shell and shell grit groups, respectively. The semen colour average score evaluated during this study were 2.35, 2.80 and 2.83, respectively in G1, G2 and G3. The average semen motility reported in this study was 47.92, 61.04 and 61.46 per cent in control, egg shell and shell grit groups, respectively. The semen qualities like semen volume, colour, sperm concentration and sperm motility were not influenced and similar results were obtained between the treatment groups. Further, serum Ca and P content and tibial characteristics were similar in all the treatment groups. The tibial calcium content was significantly ($p < 0.01$) different between the treatment groups.

The experiment II results in laying hens and roosters revealed that, free choice feeding of calcium sources (egg shell powder and shell grit) did not show any influence on feed intake between the groups. Over the course of the experiment from 33rd to 44th weeks of age, the calcium intake through feed was 4.56 and 4.45 g/day/bird in egg shell powder and shell grit group birds, respectively and not differing significantly. It was observed that the mean egg shell powder intake was 5.06 g/bird/day and provided 2.04 g of calcium/day/bird during the experimental period, without affecting feed intake between the groups. However, the calcium intake through egg shell powder feeding provides 2.04 g/day/bird and shell grit feeding provides 1.39 g/day/bird which is significantly different. The highest calcium intake was recorded at 7.10 g/day/bird in egg shell powder supplemented group at 39th weeks of age. The total calcium intake in egg shell group was significantly ($p < 0.01$) more than shell grit group, but had similar egg surface area.

The mean hen day (HD) and hen housed (HH) egg production in egg shell powder supplemented group was 57.40 and 53.04 per cent, respectively. Like that, 54.33 and 53.54 per cent, HD and HH production, respectively and they were not significantly different. The egg shell powder received birds had not shown increased egg production between the groups. The feed efficiencies for kg and dozen eggs were similar, and found average values of feed efficiency per kg egg mass in G4 and G5 were 2.84 and 2.99, respectively. Like that, average feed efficiency per dozen eggs recorded was 4.14 and 4.23 in G4 and G5, respectively.

The present experimental findings revealed that free choice of feeding of egg shell powder and shell grit had not influenced the egg weight and shape index. The albumen height, albumen index, Haugh units, yolk weight, yolk height and yolk index values were similar between the groups. Like that, egg shell quality parameters were not significantly different among the groups. The recorded values of mean specific gravity were 1.093 and 1.094 for egg shell powder and shell grit, respectively and average shell thickness recorded was 0.38 and 0.36 mm for egg shell and shell grit group, respectively. The overall mean calculated egg surface area for egg shell powder and shell grit was 67.69 and 68.24 cm², respectively. Like that calculated average egg shell weight per unit surface area (ESWSA) was 0.38 and 0.37 mg/cm² and the mean breaking strength was 2007.39 g and 2028.44 g, respectively for egg shell and shell grit groups.

The recorded overall fertility per cent was 95.24 and 94.83, respectively for egg shell powder and shell grit groups. Like that, mean hatchability per cent for 13 weeks experimental period was 84.07 and 82.33 per cent in egg shell powder and shell grit, respectively. The free-choice feeding of egg shell powder to laying hens did not affect the fertility and hatchability and the results were similar to shell grit group birds.

It was further confirmed that, calcium consumed through feed and egg shell powder were assimilated properly and maintained serum calcium values of 16.20 mg/dL and 8.40 mg/dL of phosphorus. Meanwhile, it was evident that tibia

calcium content of egg shell powder group was 20.34 per cent which was similar to shell grit fed group laying hens not differing significantly.

The profit calculated in egg shell powder group was comparatively 0.41 paise per egg more than shell grit fed group. The cost of production calculated was Rs. 7.74 and Rs. 8.15/egg in egg shell powder and shell grit group birds.

It was observed that, extra calcium intake through egg shell powder and shell grit had not influenced the semen qualities, between the groups and found similar. Hence, ICAR (2013) recommendations of 3.0 per cent calcium and 0.30 per cent available phosphorus for Gramapriya breeder layer was sufficient to maintain semen quality, because layer ration was fed to male roosters in experiment-II.

It can be concluded that egg shells (more than 2 mm) can be used as a calcium source in Gramasree breeders without affecting the semen quality, egg production, fertility and hatchability, with low production cost.

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**PERFORMANCE OF GRAMASREE BREEDER BIRDS FED WITH
DIFFERENT SOURCES OF CALCIUM**

**SABARINATH E
(19-MVP-26)**

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

MASTER OF VETERINARY SCIENCE

(Animal Nutrition)

2021

**Faculty of Veterinary and Animal Sciences
Kerala Veterinary and Animal Sciences University**



**DEPARTMENT OF ANIMAL NUTRITION
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
POOKODE, WAYANAD – 673 576
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ABSTRACT

A feeding trial was conducted for 90 days to assess the effects of supplementing free-choice feeding of egg shell powder and shell grit on semen and egg production characteristics of Gramasree birds. The egg shells were collected from the local market, washed and the membrane was removed before being powdered into a coarse particle size of more than 2 mm. Similarly, shell grit with more than 2 mm particle size was used. In experiment-I, 18 Gramasree roosters of the same age group were randomly assigned to three experimental groups (G1, G2 and G3) with six birds in each group and separately housed in male cages. In experiment II, 360 Gramasree breeder hens and 36 roosters (Gramasree) of 32-weeks of age were randomly allotted to G4 and G5 with three replicates and 60 birds of female and six birds of male in each replicate and housed in deep litter. Experimental layer breeder ration (control) was prepared as per ICAR, (2013) recommendations and fed to birds in cages and deep litter.

The free-choice feeding of egg shell powder and shell grit was given to male Gramasree birds in G2 and G3 groups, respectively, and found that semen qualities like semen volume, colour, sperm concentration and sperm motility were not influenced and similar results were obtained between the treatment groups. The serum Ca and P content and tibial-ash were similar in all the treatment groups. The tibial calcium content was significantly ($p < 0.01$) different between the treatment groups. It was observed that average calcium intake from egg shell powder and shell grit intake was only 0.26 and 0.30 g/bird/day, respectively during the experimental period and it did not differ significantly among treatments. However, their feed intake during the period was significantly different ($p < 0.01$) and body weight also differ ($p < 0.01$) between the treatment groups.

The free-choice feeding of egg shell powder and shell grit to Gramasree laying hens was studied and mean values for hen day egg production (57.40 per cent) and hen housed egg production (54.33 per cent) from 32 to 44th weeks of age in egg shell powder was similar to shell grit treatment group. The mean hatchability recorded in the egg shell powder group was 83.83 per cent and in the shell grit group was 82.45 per cent. Like that, the fertility rate was 94.87 and 94.58 per cent for G4 and G5 group birds. The mean feed efficiency to produce a dozen eggs was 4.14 and 4.23 in egg shell

powder and shell grit group birds, respectively. The mean value of external and internal egg quality parameters was not significantly different among the groups.

It was observed that the mean egg shell powder intake was 5.06 g/bird/day and provided 2.04 g of calcium/day/bird during the experimental period, without affecting feed intake between the groups. Further, the calcium content in layer ration which was usually fed to male breeder Gramasree birds was sufficient to maintain semen quality. Like that, free-choice feeding of egg shell powder to laying hens did not affect egg production, fertility and hatchability and similar to shell grit group birds. The cost of production per egg calculated was Rs. 7.74 in egg shell powder and Rs. 8.15 in shell grit group birds.

In conclusion, egg shells (mean particle size of more than 2 mm) can be used as a calcium source in male and female Gramasree breeders without affecting the semen quality. egg production, fertility and hatchability, with low production cost.

KERALA VETERINARY AND ANIMAL SCIENCES UNIVERSITY
Faculty of Veterinary and Animal Sciences
PROGRAMME OF RESEARCH WORK FOR THESIS FOR MASTERS DEGREE

1. Title of thesis

Performance of Gramasree breeder birds fed with different sources of calcium

2. a) Title of the departmental / KVASU research project of which this forms a part

Nil

(b) Code No. if any, and order by which the departmental/KVASU research project is approved

Nil

3. a) Name of student

Sabarinath E

b) Admission No

19-MVP-26

c) Name of the Discipline

Animal Nutrition

4. a) Name of Major Advisor

Dr. S.Senthil Murugan

b) Address

Assistant Professor,
Department of Animal Nutrition,
College of Veterinary and Animal
Sciences, Pookode, Wayand- 673 576

5. Objectives of the study

1. Assess the effect of supplemented egg shell powder and shell grit on semen quality of Gramasree roosters

2. Assess the effect of supplemented egg shell powder and shell grit on egg production, egg quality, fertility and hatchability of breeder hens

3. Techno-economics of supplementing egg shell powder and shell grit on egg production

6. Practical/Scientific utility

Egg shell consists of 98.2 per cent calcium carbonate and protects the egg contents against environmental contamination on handling and breaking during transportation. The particle size, source, supplementation level and feeding time of calcium affect the egg shell quality.

In this context, aim of our present study is to evaluate the effect of supplementing dried egg shell powder as cheapest source of calcium and compare against shell grit on egg production performance, semen characteristics and hatchability in Gramasree breeder birds.

7. Important publications on which the study is based

Safaa *et al.* (2008) studied the effect of calcium supplementation at different levels by different sources in lohmann brown layers and found that hens in late egg production stage required 4.08 per cent of calcium in the diet to maintain productivity and shell quality. Whereas, 40 per cent of fine limestone powder replaced by oyster shell had not improved the egg shell quality and tibia characteristics.

Kanyinji *et al.* (2010) found that feeding dietary calcium at 3.1 per cent level had shown enhanced seminal calcium level, sperm motility, thermo-tolerance and cryo-survivability in Barred Plymouth Rock roosters, however at 5.4 per cent calcium level had shown deleterious effect on sperm quality.

Gongruttananun (2011) conducted a trial with supplementing egg shells in Rhode Island Red layers and roosters and recommended that egg shells could be included in breeder ration at 9.06 per cent in place of limestone powder without any difference in the semen volume, sperm concentration per ml and sperm number per ejaculation and effect on egg

productive performance, fertility and hatchability.

Pizzolante *et al.* (2011) recommended that replacing 50 per cent of fine limestone powder with coarse limestone or oyster shell significantly improved egg shell quality with 3.8 per cent of dietary calcium.

Khalil *et al.* (2012) reported that cocks fed with two and three per cent calcium levels had superior forward sperm motility, live sperm and fertility per cent compared with those fed with one per cent calcium.

Ahmed *et al.* (2013) found that limestone supplementation as calcium source had shown better egg production, feed intake and feed conversion efficiency compared to oyster shell. However, no changes were reported in egg shell quality.

Olgun *et al.* (2015) suggested that egg shell used as calcium source lowered calcium excretion as compared to limestone in layer diets without any detrimental effects on performance and egg shell quality.

Lertchunhakiat *et al.* (2016) mentioned that grounded egg shell can be used as calcium source in breeder cock

diet with normal feed intake and semen quality.

In brown colored egg laying hens egg shell quality was improved by feeding 4.7 per cent of calcium in the diet (An *et al.*, 2016).

Saki *et al.* (2019) studied on particle size of calcium source and recommended that oyster shell with 2-4 mm particle size replacing 50 per cent of fine oyster shell powder improved the physical quality of eggs.

8. Outline of the technical programme Experiment I

Eighteen Gramasree roosters of same age group will be selected for semen quality studies based on their body conformation (Aviagen, 2018) from Poultry Farm, Instructional Livestock Farm Complex (ILFC), College of Veterinary and Animal Sciences (CVAS), Pookode. The roosters will be randomly assigned to three experimental groups (G1, G2 and G3) with six birds in each group and separately housed in male cages.

Roosters in experimental cages will be fed with layer breeder feed (control). The egg shell powder and shell grit will be given as additional calcium source as

mentioned below in the table (Experiment I)

The experimental layer breeder feed (control) will be prepared as per ICAR, (2013) recommendations.

Groups	Experiments
Experiment- I	
G1	Layer breeder feed (control) with 3 per cent calcium to roosters in cages
G2	Layer breeder feed (control) + extra egg shell powder (20 g) to roosters in cages
G3	Layer breeder feed (control) + extra shell grit (20 g) to roosters in cages

Daily feed and calcium intake at weekly interval will be studied for experimental period of 90 days.

Four roosters from each group housed in cages will be randomly selected for semen quality studies (Peters *et al.*, 2008) at weekly interval. The semen will be collected at 10.00 to 10.30 am by abdominal massage method (Burrows and Quinn, 1937).

Experiment II

Three Hundred Sixty Gramasree breeder hens of same laying period and

36 roosters (Gramasree) of same age group will be selected based on their body conformation (Aviagen, 2018).

They will be randomly allotted to two experimental groups (G4 and G5) with three replicates and 60 birds of female and six birds of male in each replicate and will be housed in deep litter system.

Experimental layer breeder feed (control) will be prepared as per ICAR, (2013) recommendations and will be given to roosters and layers.

Groups	Experiments
Experiment-II	
G4	Layer breeder feed (control) to roosters and layers + extra egg shell powder (20 g) in male and female feeders separately.
G5	Layer breeder feed (control) to roosters and layers + extra shell grit (20 g) in male and female feeders separately.

The egg shell powder and shell grit will be provided as extra calcium source. Separate male feeders will be provided for the roosters.

The sundried egg shells without shell membrane will be dried in hot air oven at 120° C for 24 h and made into powder by laboratory mill. The egg shell particles which are not passing through two mm Indian standard (IS) sieve will be used for feeding the birds. Like that, shell grit available in local market above two mm particle size will be used as additional calcium source in this study.

Data on egg production, egg weight, shape, fertility hatchability will be collected. Daily feed and calcium intake at weekly interval will be studied and experiment will be continued for 90 days.

At end of experimental period, four roosters housed in cages and four layers in deep litter will be randomly selected to estimate tibial ash and calcium content (Gongruttananun, 2011b).

Totally, 36 eggs will be randomly collected from each group in a week on alternate days and will be stored in cool room for internal and external egg quality studies (Rath *et al.*, 2015).

On 18th day of setting, all the eggs will be candled to find fertility per cent. The hatchability per cent will be calculated for every hatch (Patra *et al.*, 2016).

Chemical composition of the layer breeder feed will be analysed as per AOAC (2016).

The economics of supplementing egg shell powder and shell grit will be studied. Data on various parameters will be analysed statistically using SPSS version 24.0.

9. Main items of observations to be made

1. Egg characteristics

- a) Egg shell thickness (mm)
- b) Shape index
- c) Egg specific gravity
- d) Haugh unit
- e) Yolk colour

2. Semen characteristics

- a) Semen volume (ml)
- b) Semen colour
- c) Sperm motility (%)
- d) Sperm concentration

3. Tibial - ash and calcium (%)

4. Serum Calcium and Phosphorus at weekly interval

5. Fertility and hatchability (%)

6. Chemical composition of feed, egg shell powder and shell grit

10. Facilities required

a) Existing

The facilities available in the Department of Animal Nutrition at Pookode and Mannuthy, Department of Animal Reproduction, Gynaecology and Obstetrics at Pookode, Poultry Farm, ILFC, CVAS, Pookode, Wayanad will be utilised for the study.

b) Additional facilities required
Chemicals and Biologicals

11. Duration of study

Four semesters

12. Financial estimate

Cost of feed additive	Rs.23,000
Contingencies	Rs.2,000

Total	Rs. 25,000
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Signature of student

Project coordination group to which the proposal is to be placed

Poultry and Duck

Signature of Major Advisor

Place: Pookode

Date: 21.12.20

Name and signature of other members of the Advisory Committee

1. Dr. Biju Chacko
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14. Time frame of work

Semester I

1. Collection of literature
2. Planning of programme of research work
3. Preparation of synopsis

Semester II

1. Collection of literature
2. Procurement of birds, feed and feed additives
3. Conducting the experiment

Semester III

1. Conducting the experiment
2. Collection of data
3. Analysis of samples

Semester IV

1. Statistical analysis of data and interpretation of result
2. Preparation and submission of thesis

CERTIFICATE

Certified that the research project has been formulated observing the stipulations laid down under the Prevention of Cruelty to animals Act (Amendment, 1998).

Place: Pookode

Date: 21.12.20

Dr. S. Senthil Murugan

Major Advisor

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5. Permanent Address: 32, TVS nagar, Nagore main road,
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